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BRAIN-TARGETED CALCIUM CHANNEL ANTAGONIST ANTICONVULSANT AGENTS

BY

SAI HAY YIU

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled BRAIN-TARGETED CALCIUM CHANNEL ANTAGONIST ANTICONVULSANT AGENTS submitted by SAI HAY YIU in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Pharmaceutical Sciences (Medicinal Chemistry).

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ABSTRACT

The syntheses and pharmacological evaluation of novel 1,4-dihydropyridines, and prodrugs thereof, have been investigated as anticonvulsants.

The Hantzsch condensation of substituted-benzaldehydes with alkyl 3-aminocrotonates and alkyl acetoacetates afforded the reference drug nimodipine (**38**) and various 3-substituted-alkyl 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (**96, 102 - 104, 110, 111, 137, 139, 148, 150, 151, 154 - 156, 164**). 3-Ethyl 5-methyl 1,4-dihydro-2-[(2-hydroxyethoxy)methyl]-6-methyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**128**) was obtained by the condensation of methyl 2-(2,3-dichlorobenzylidene)acetoactate (**87**) with ethyl 4-(2-hydroxyethoxy)acetoacetate (**127**) and ammonium acetate. Hydrogenation of the 3-nitrophenyl substituent of (**38**) and (**156**) to the amino analog followed by reductive-amination using formaldehyde and sodium cyanoborohydride afforded the 4-(3-dimethylaminophenyl)-1,4-dihydropyridines (**152**) and (**153**). Methylation of the dimethylamino moiety of (**139**) with iodomethane yielded the quaternary ammonium salt (**140**). High-purity felodipine (**39**) (99.3% purity) was prepared using an efficient β-elimination reaction of a 2-cyanoethyl ester using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

To enhance the delivery of calcium channel antagonist drugs to the brain, a variety of chemical delivery systems were coupled to the alkyl chain of a C-3 alkyl ester substituent such as valproate (**98 - 101**), valerate (**108 - 109**), the Bodor chemical delivery system 1-methyl-1,4-dihydropyridyl-3-carbonyloxy (**118 - 120, 131**), 4,4-*bis*-(3-

methyl-2-thienyl)-3-butenyl (144), 4,4-*bis*-(*o*-tolyl)-3-butenyl (148) and 1-[4-(*p*-fluorophenyl)piperazinyl] (137).

It was assumed that the 1,4-dihydropyridyl moiety of the Bodor chemical delivery system would be oxidized in the brain to form a pyridinium salt which would prevent egression of the drug from brain. Hydrolysis of the pyridinium ester would liberate the active dihydropyridine anticonvulsant agent in brain.

Anticonvulsant activities for most of the compounds synthesized (38, 39, 98 - 103, 108, 109, 110, 111, 118 - 120, 128, 131, 137, 139, 140, 144, 148, 150 - 155) were determined using the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scMet) screens. The majority of 1,4-dihydropyridines coupled to the Bodor chemical delivery system were found to be inactive. In contrast, the felodipine analog (118) exhibited activity with an onset of action at 4 hours.

Calcium channel antagonist activity (IC_{50}) was determined by measuring the ability of the test compound to inhibit the tonic component of the muscarinic-induced contractile response of guinea pig ileal smooth muscle.

These brain-targeted dihydropyridines offer some protection against seizures. A comparison of their calcium channel antagonist activities with anticonvulsant activity indicates that calcium is not likely the sole factor for epileptogenesis.

In vitro incubation of felodipine (39), and its prodrug (118), with rat plasma or brain homogenate and subsequent quantitative HPLC analyses indicated that felodipine was very stable in both biological media, whereas (118) was more stable in rat plasma than in brain homogenate.

In vivo biodistribution studies for (39) and (118) in rats indicated that both compounds were rapidly taken up in brain. However, compound (39) had exited the brain by 1 hour postadministration, while (118) was oxidized to a pyridinium salt which was retained in the brain for up to 4 days after drug administration. However, liberation of the active drug (102) by hydrolysis of the pyridinium salt ester was very slow. This result offers an explanation for the slow onset of action of (118) in the anticonvulsant screens, and possibly the lack of activity for other dihydropyridines coupled to the Bodor chemical delivery system.

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Last but not the least, I have to thank God in whom all fullness dwells. In these years of fathoming knowledge, I couldn't help but always stop and consider God's wonders as Job once said, "I know that you can do all things..... things too wonderful for me to know" (Job 42:2 - 3).

11th January, 1995

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14 Jan. 1995

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Dedicated to

Diana, my wife

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LIST OF ABBREVIATIONS

AEDs	antiepileptic drugs
ap	antiperiplanar
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
AV	atrial-ventricular
BBB	blood brain barrier
bp	boiling point
cAMP	cyclic adenosine monophosphate
CCA	calcium channel antagonist
CDS	chemical delivery system
CNS	central nervous system
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-dicyclohexylcarbodiimide
DHP	1,4-dihydropyridine
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
ED_{50}	effective dose of a drug that produces 50% of activity
EDCI	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
EEG	electroencephalogram
EPSP	excitatory post-synaptic potential
EtOH	ethanol
FDA	Food and Drug Administration
GABA	γ-aminobutyric acid
GABA-T	γ-aminobutyric acid transaminase
GAD	glutamic acid decarboxylase
GPILSM	guinea pig ileal longitudinal smooth muscle
^{1}H NMR	proton nuclear magnetic resonance

HPLC	high performance liquid chromatography
Hz	Hertz
IC_{50}	concentration of a drug that produces 50% inhibition
ILEA	International League Against Epilepsies
ip	intraperitoneal injection
IPSP	inhibition post-synaptic potential
IR	infra-red
LD_{50}	lethal dosage - dose of drug that produces 50% fatality
M	molar concentration
MES	maximal electroshock seizure test
mg/kg	milligram per kilogram
mM	millimolar concentration
mp	melting point
NAD (NADH)	nicotinamide adenosine dinucleotide
NIH	National Institute of Health
NMDA	N-methyl-D-aspartate
NOE	Nuclear Overhauser Enhancement
PEMA	phenylethylmalonamide
psi	pounds per square inch
PTZ	pentylenetetrazole
rpm	revolutions per minute
ROC	receptor-operated calcium channel
R_T	retention time
SA	sino-atria
SAR	structure-activity relationship
scMet	subcutaneous pentylenetetrazole (Metrazol®) seizure threshold test
S_N2	nucleophilic substitution bimolecular
sp	synperiplanar
t-BuOH	*tertiary* butanol
THF	tetrahydrofuran

TLC	thin layer chromatography
UV	ultraviolet
VOC	voltage-operated calcium channel
WHO	World Health Organization

1.0.0.0.0 INTRODUCTION

The word 'Epilepsy' is derived from the Greek word 'Epilambanein' which means 'to seize'.[1] Epilepsy is defined as "A chronic brain disorder of various etiologies characterized by recurrent seizures due to excessive discharge from cerebral neurons associated with a variety of clinical and laboratory manifestations".[2] According to this definition, a single seizure should not be considered as epilepsy unless the seizure is recurrent.

Although the prevalence of epilepsy in industrialized countries is between 3 - 9 per 1000 population (~ 1 %), it is as high as 57 per 1000 population (5.7%) in some developing countries.[3] The estimated number of epileptic patients in the U.S. alone is about 1.4 million.[4]

1.1.0.0.0 EPILEPSY INFORMATION

1.1.1.0.0 Etiologies of Epilepsy

While the etiologies of seizures are diverse including head trauma, cerebral infection, tumor or toxic disturbance, the cause of seizures in 30 - 40% of patients remains unknown.[3] However, a recent approach using genetics and molecular biology supplements the molecular basis for inherited epilepsies which are estimated to account for 20% of epileptic cases.[5] In fact, recent studies have identified mutant genes that are responsible for some familial epilepsies.[6] It is anticipated that further research in this field will provide mechanistic information pertaining to the different types of seizures.

1.1.2.0.0. Prognosis of Epilepsies

Traditionally, epilepsy has been regarded as a chronic disease for which the prognosis is usually poor. However, Sander[7] in his review of historical prognosis has shown a significant advancement in the last 30 years. With the introduction of new antiepileptic drugs, as many as 70 - 80% of epileptic patients can expect permanent remission of seizures. About 20 - 30% of individuals are regarded as 'chronic epileptics' that are refractory to current medical treatment. Similar findings were reported by

Annegers *et al* [8] and Goodridge *et al.*[9] Goodridge observed that epileptics having partial or mixed seizures are the least responsive to treatment and have a less favorable prognosis. Although there has been a remarkable increase in the use of surgery as an alternative mode for correcting intractable epilepsies, starting in the late 1980's, the number of surgeries performed has not kept pace with the demand. For example, of the 250,000 epileptic patients in the U.S. who could be considered as suitable candidates for surgery, only about 300 surgeries were performed annually.[10] This low number is attributed to the fact that many factors have to be considered such as safety, potential success of the surgical intervention, evaluation of the patient as a suitable candidate and the surgical outcome of such treatment.[11]

1.1.3.0.0.0. Psychosocial Aspects of Relevance to Epileptic Individuals

Although sudden death occurs rarely during or immediately after seizures, it is often difficult to establish the cause of death as being due to seizures. Thus, many deaths are due to accidents, seizure related diseases or sudden unexpected death. A recent report concerning the mortality of severe epileptic patients in residential care found that the mortality rate of epileptic individuals was almost twice that of the general population.[12] This finding suggests most intractable epileptic individuals must live with the anxiety of a potential seizure mortality.

A greater concern, apart from the risk of death, is the quality of life for epileptics. Other than the psychological acceptance of their self-image, epileptics also suffer from the impairment that arises from the seizure itself and the side-effects induced by antiepileptic drugs. Many psychosocial factors,[13] such as schooling, routines of daily life, occupation, interpersonal relationships, housing and driving,[14] often result in social withdrawal. Consequently, there is an urgent need for the development of effective antiepileptic drugs for the treatment of this group of intractable epileptic individuals.

1.1.4.0.0.0 Classification of Epileptic Seizures

Since there are many clinical manifestations of epileptic seizures, it is essential that their classification employs a common language easily understood by both clinical and research personnel. With this in mind, the International League Against Epilepsies (ILAE) devised the first proposal for seizure classification in 1969.[15] However, the subsequent introduction of advanced medical technologies such as the simultaneous videotaping of the electroencephalogram (EEG) and the clinical manifestation of seizures, prompted the need for revision. With this purpose in mind, a commission for seizure classification was formed under the ILAE in 1979. In 1981, a revised classification based on clinical manifestations was developed.[16] Despite the fact that this classification possesses limitations, and that several revisions based on syndromes have since been proposed,[17, 18] it is still the most widely used and useful classification guide.

In principle, seizures can be classified as partial or generalized (see Table 1). In partial seizure, the onset of the seizure is localized in one area of the cerebral cortex and hence it is also referred to as a focal or local epileptic seizure. On the other hand, a generalized seizure is generalized from the start with the involvement of both hemispheres. In addition, a partial seizure may further be classified as simple or complex depending on whether consciousness is impaired. Apart from classifying different types of seizures, the term "status epilepticus" is defined as "a seizure which persists for a sufficient length of time or is repeated frequently enough that recovery between the attacks does not occur."

Sander[7] classified epileptic individuals into four prognostic groups, the excellent, good, uncertain and bad. Those patients with childhood absence or tonic-clonic seizures are classified as good since their seizures are easily controlled by antiepileptic drugs. Focal motor, myoclonic and some infancy seizures are classified as bad since these latter epileptics are more prone to elicit seizures despite medical treatment.

Table 1 Classification of Epileptic Seizures

This simplified table does not include the EEG expressions. More detailed information is provided in reference #16.

Types of seizures	Clinical manifestation
I. Partial seizures	
A. Simple partial seizures	Consciousness is usually not impaired.
(i) Focal motor or Jacksonian seizures	Any part of the body may be involved and it is manifested by a sequence of body movement defined as a Jacksonian march.
(ii) Somatosensory seizures	Apart from epileptic march, the individual also experiences sensory hallucinations which may be visual, auditory, olfactory or gustatory.
B. Complex partial seizures It may be triggered by a simple partial or a complex partial seizure from the onset.	Consciousness is impaired. Automatism or coordinated involuntary motor activity during/after seizure usually occurs.
II. Generalized seizures	
A. Absence (petit mal seizures)	A sudden unresponsive state may interrupt the patient's normal activity for a few seconds, or up to 30 seconds. Automatism or clonic movement may occur.
B. Tonic-clonic seizures (grand mal seizures)	As its name indicates, this seizure type is characterized by a sudden tonic contraction of all muscle groups causing the individual to fall. This event is then followed by a clonic convulsion which may last a period of time before regaining consciousness.
C. Myoclonic seizures	It is marked by a sudden contraction of muscles.

1.2.0.0.0.0. THE MECHANISMS OF EPILEPTOGENESIS

1.2.1.0.0.0. The Principles of Transmission in Neurons

Neurons are able to maintain an asymmetric distribution of ions across its plasma membrane. There is an extracellular excess of Na^+, Cl^- and Ca^{2+} ions while the intracellular environment is marked by an excess of K^+. This uneven distribution of ions can generate an electric potential across its membrane. When the neuron is at rest (i.e. not transmitting any signal), there is a potential difference of -70 to -90 mV (see Figure 1). This negative electrical potential, called a resting potential, suggests the K^+ current dominates over the Na^+ current. Diffusion of K^+ into the extracellular space would leave a net negative intracellular charge, and alternatively a negative extracellular charge can be generated for the Na^+ current. Similarly, a flow of anions such as Cl^- into the cell can also maintain a negative resting potential.

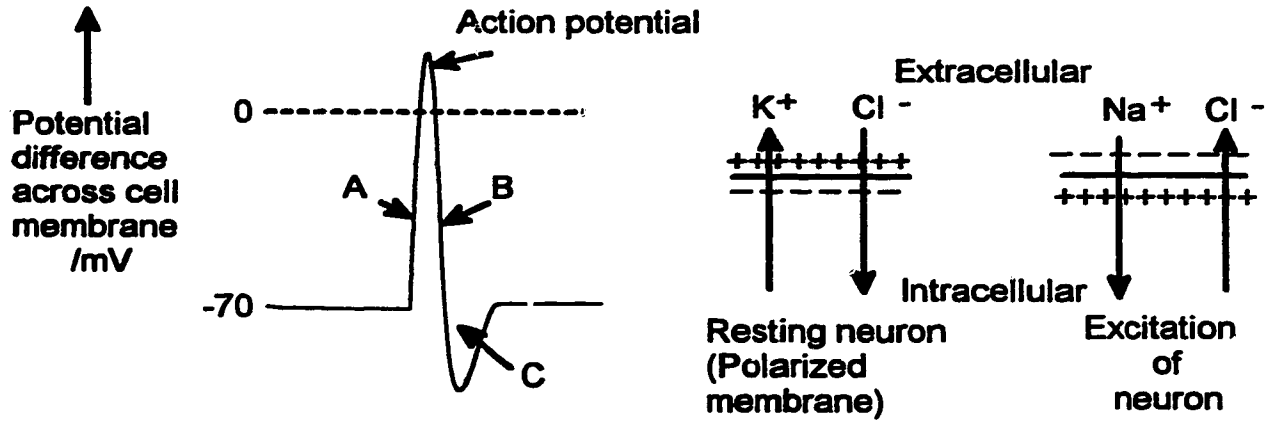


Figure 1. The change in electrical potential of a neuronal membrane from the resting to excitation state. The middle and right diagrams indicate the dominating ionic currents across the cell membrane in resting and excitation states, respectively.

Excitation of the neuron, due to a stimulus from neurotransmitter release from a presynaptic neuron, results in a Na^+ influx into the cell causing a depolarization as shown by A in Figure 1. The resting potential is then replaced by a positive action potential. This action potential, which is excitative in nature, is sometimes called the

excitation post-synaptic potential (EPSP). This depolarized state is immediately reversed by the exclusion of Na^+ and K^+ from the cytoplasm and the concurrent influx of Cl^- (B). Due to excessive influx of Cl^-, the membrane is hyperpolarized (C) and the electrical potential is called an inhibition post-synaptic potential (IPSP).

Although Ca^{2+} does not participate in the EPSP, due to its low conductivity, it plays an important role in the nerve ending when neurotransmitters are released. As the impulse reaches the nerve-ending, voltage-operated Ca^{2+} channels are activated causing an influx of Ca^{2+}. This influx of Ca^{2+} is essential for a number of events. Thus, the entry of Ca^{2+} can activate an efflux of K^+ to restore the resting potential of the neuron.[19] Perhaps the most important function is a triggering of neurotransmitter release from its intracellular store. Although the mechanism for this process is still controversial, it has been reported that neurotransmitter release can be augmented by injecting Ca^{2+} into squid nerves.[20]

1.2.2.0.0.0. Mechanisms of Focal Epileptogenesis

Sander[7] pointed out that epilepsy should not be considered as a single disease. Rather, it is a collection of syndromes marked by recurrent seizures of many etiological origins. It is therefore difficult to provide a generalized scheme to explain the mechanism of seizure induction.

Recent studies investigating the mechanism of epileptogenesis using animal models have shed some light on the causes underlying partial seizures. The mechanism giving rise to generalized seizures, however, remains unclear. Treiman[21] pointed out that the extent of horizontal spread of seizure activity in the cerebral cortex determines whether a seizure is simple partial, complex partial or secondary generalized.

In a variety of experiments using hippocampal slices and neocortex, Prince[22] showed that there are three requirements for the development of focal seizures, namely,

(i) Certain pace-maker cells which can generate intrinsic burst discharges.

(ii) The mechanism of inhibition is lost (disinhibition).

(iii) The presence of excitatory synaptic circuitry which facilitates the synchronization of a population of neurons.

1.2.2.1.0.0. The Generation of Burst Discharges in Neurons

The mechanism underlying the burst-firing of pace-maker neurons has been studied. Using hippocampal pyramidal cells and the patch-clamp technique, Johnson and Hablitz[23] showed that the main event is effected by a slow inward Ca^{2+} current. The initial burst discharge event is similar to the depolarization of neurons by an influx of Na^{+}. However, the action-potential that follows is mediated by a voltage dependent Ca^{2+} current which serves to activate the Ca^{2+} threshold. The influx of Ca^{2+} would then activate the outward K^{+} current which then facilitates hyperpolarization of the membrane.

1.2.2.2.0.0. Mechanism of Inhibition

Prince[22] showed that direct excitation of neurons is not responsible for the epileptiform in the electroencephalogram (EEG) as expected for seizures. This result is due to the fact that a protection mechanism is operative. The excitation postsynaptic potential (EPSP) triggers the inhibition postsynaptic potential (IPSP) which then hyperpolarizes the membrane. Thus, the inhibitory neurotransmitter γ-aminobutyric acid (GABA), on binding to the $GABA_A$ receptor, opens the Cl^{-} channel. Influx of Cl^{-} would then hyperpolarize the membrane. In fact, blocking the action of GABA or GABA receptors by agents such as pentylenetetrazole (PTZ) or bicuculline would generate seizures.[24]

1.2.2.3.0.0. Excitatory Synaptic Circuitry

The typical EEG epileptiform in epileptic individuals is characterized by a large number of spikes, and each spike represents the synchronization for a large number of neurons that have the same action potential induced by burst-firing.[22] This synchronized activity is facilitated by the presence of excitatory synaptic circuitry. The neurotransmitter for excitation is mediated by acidic amino acids such as L-glutamate and possibly aspartate. Recent studies have identified receptor sub-types for excitatory amino acids[25] which are named after the agonists which activate them, viz N-methyl-D-aspartate (NMDA), quisqualate and kainate. Each receptor type is believed to couple

with an ion channel. The NMDA receptor, which is by far the most important, activates Na^+ and Ca^{2+} channels.[26,27] Injection of N-methyl-D,L-aspartate (NMDLA) has been found to generate seizures in animals[28] indicating the important role of excitatory neurotransmitters in epileptogenesis.

1.2.2.4.0.0. Pathological Processes in Epileptogenesis

It is obvious that the three requirements summarized in the previous section (1.2.2.0.0.0.) play an important role in epileptogenesis. However, the question of how a pathological modification can give rise to a convulsive disorder has not yet been fully elucidated. In order to provide a plausible explanation for the irregular burst-firing that occurs in neurons, Prince[22] postulated that up-regulation of ionic channels following neuronal injury could be the source of pace-maker activity. In particular, an increase in the number of Na^+ channels would lower the threshold of intrinsic burst-firing.

Recent studies indicated that excessive stimulation of NMDA receptors by excitative amino acids can cause neuronal cell death.[29-30] This is probably due to excessive influx of Ca^{2+} and water across the membrane which causes considerable cell damage.[31] In studies pertaining to cell death in the hippocampus, Shin and McNamara[5] put forward two hypotheses, i) the Dormant Basket Cell Hypothesis, and ii) the Mossy Fiber Sprouting Hypothesis, to explain the hyperexcitability and loss of inhibition in neurons.

Basically, the Basket Cell is an interneuron which, upon activation by the Mossy Cell, can produce inhibition. However, excessive activation of the latter would result in its death leaving the Mossy Cell in a dormant state. The disinhibition that follows would result in seizure initiation.

The second hypothesis is similar. Death of the Mossy Cell would lead to sprouting of the axons (Mossy fiber) which in turn would increase the number of excitatory synapses (recurrent excitatory synaptic circuits). Excessive excitation would facilitate synchronization of a population of neurons.

The mechanisms described represent some of the many possible explanations that can be used to explain the cellular or molecular basis for seizure generation. In the

future, more advanced techniques particularly at the cellular and molecular level and information from genetic studies will be used to test the different hypotheses proposed.

1.3.0.0.0.0. ANTIEPILEPTIC DRUGS

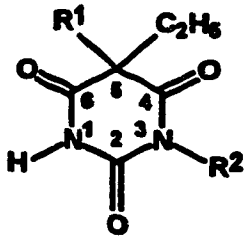
There has been a renewed interest to develop more efficacious antiepileptic drugs (AED). Pharmaceutical companies now screen compounds that show Central Nervous System (CNS) activity for antiepileptic activity.[32] There are now about 14 classes of compounds that are either available on the market, or that are being tested as potential AEDs.

1.3.1.0.0.0. Antiepileptic Drugs in Current Clinical Use

1.3.1.1.0.0. The Bromides

Potassium bromide, the first AED was introduced by Locock[33] in 1857. Despite the fact that its use was reduced when phenobarbital became available in 1912, and later by other AEDs, potassium bromide is still very useful for the treatment of intractable generalized tonic-clonic seizures.[34] Its major disadvantage is the appearance of toxic psychotic side-effects at the required therapeutic plasma concentration range of 10 - 20 mM.

1.3.1.2.0.0. The Barbiturates



(1) $R^1 = Ph$, $R^2 = H$ Phenobarbital

(2) $R^1 = Ph$, $R^2 = CH_3$ Methylphenobarbit

(3) $R^1 = C_2H_5$, $R^2 = CH_3$ Metharbital

Phenobarbital (1), which was introduced in 1912, was the first organic anticonvulsant drug used. Subsequently, methylphenobarbital (2) and metharbital (3) were introduced as AEDs.

It has been shown that increasing the length of the C-5 alkyl substituent can convert barbiturates to convulsants[35] suggesting that a C-5 ethyl moiety is the maximal length for anticonvulsant activity.

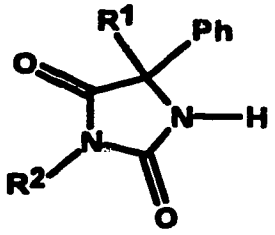
Of the three barbiturates listed, phenobarbital is well tolerated and it is used for the treatment of clonic-tonic and simple partial seizures. However, it is not very effective against absence seizures. No serious side-effects are associated with its use at therapeutic dosages except for sedation which is usual at the onset of administration. Apart from this, phenobarbital can induce hepatic microsomal enzyme activity[36] that enhances the metabolism of other drugs or endogenous compounds.

The mechanism by which phenobarbital exhibits anticonvulsant activity has been investigated.[37-38] Phenobarbital increases the GABA chloride current in neurons indicating anticonvulsant activity is probably due to an enhancement of GABA inhibition.

Although metharbital (3) is rarely used, the use of methylphenobarbital is increasing[39] as it is less sedative and it enters the brain faster than phenobarbital. The anticonvulsant effect of methylphenobarbital is probably due to its biotransformation to phenobarbital by N-demethylation.

1.3.1.3.0.0. The Hydantoins

The most useful compound in the hydantoin class is phenytoin (Dilantin®) (4), which was introduced in 1938. Despite the wide spectrum of undesirable side-effects[40] that include vertigo, tremor, ataxia and headache, phenytoin is effective for the treatment of generalized tonic-clonic and partial, but not absence seizures.



(4) $R^1 = Ph$, $R^2 = H$ Phenytoin

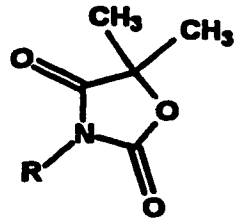
(5) $R^1 = C_2H_5$, $R^2 = CH_3$ Mephenytoin

(6) $R^1 = H$, $R^2 = C_2H_5$ Ethotoin

The mechanism of action of phenytoin is considered to be 3-fold. First, it is very effective in abolishing the pace-maker, or burst-firing, activity of neurons by blocking the sodium channel in a voltage-dependent manner.[27] Inhibition of sodium influx results in a stabilization of the membrane potential. A second remarkable action is its effect on Ca^{2+}. It was reported that phenytoin blocks Ca^{2+} influx in depolarized synaptosomes and eliminates the increase of Ca^{2+} accumulation which usually occurs upon stimulation.[41,42] Whether this effect is caused by inhibition of Na^+ or a direct effect on the Ca^{2+} channel is not clear. Inhibition of Ca^{2+} would provide a significant reduction in the excitation postsynaptic potential by blocking the release of excitatory neurotransmitters. As a result, spread of the discharge from the epileptic focus would be prevented. Finally, phenytoin was found to enhance the active transport of Na^+ and K^+ by activating the enzyme Na^+, K^+-ATPase.[43] Activation of this enzyme in synaptosomes or glia would enhance the exchange of intracellular Na^+ for extracellular K^+ which accumulates after excitation. In particular, the removal of K^+ would reduce the hyperexcitability of neurons. This fact is further supported by the finding that abnormalities in Na^+,K^+-ATPase phosphorylation plays an important role in focal epileptic seizures.[44]

In addition to phenytoin, there are two other compounds in the hydantoin class. Mephenytoin (5), like phenytoin, is effective for controlling tonic-clonic seizures. However, its use is limited by undesirable toxicities that include pancytopenia and irreversible aplastic anemia.[45] Ethotoin (6) is seldom used due to its low efficacy despite the fact that, unlike phenytoin, it is free from serious side-effects.

1.3.1.4.0.0. Oxazolidinediones



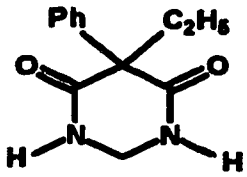
(7) R = CH_3 Trimethadione

(8) R = H Dimethadione

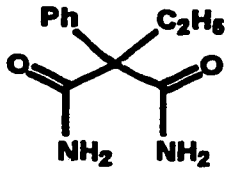
Trimethadione (7), an oxazolidinedione introduced in 1946, was the first effective drug for controlling absence seizures. Trimethadione is metabolized extensively to dimethadione (8) by N-demethylation.[46] The metabolite (8) and the parent compound (7) both possess anticonvulsant properties although they exhibit different pharmacokinetic and physicochemical properties.[47] The mechanism of action by which (7) and (8) exhibit their anticonvulsant effect is not very clearly defined. Trimethadione and dimethadione are believed to suppress both pre- and post-synaptic transmission[48,49] although the effect on GABA levels is not very pronounced.[50] Recent studies[51, 52] indicated that the metabolite (8) inhibits the low threshold calcium current in thalamic neurons in a manner similar to that of ethosuximide. Whether this effect is responsible for synaptic inhibition is not clear, but its inhibition suggests a low threshold Ca^{2+} current may be linked to the pathogenesis of absence seizures.

Despite the fact that trimethadione is an effective anti-absence drug, it is no longer a drug of choice. A wide range of toxic side-effects[53] such as teratogenesis, hemeralopia (day blindness and photophobia), sedation, depression of bone-marrow and nephrotic syndrome are associated with its use. Accordingly, it has been replaced by ethosuximide and valproate which are less toxic.

1.3.1.5.0.0. Primidone



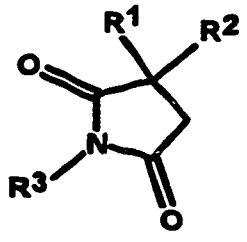
(9) Primidone



(10) Phenylethylmalonamide

Primidone (9) is an effective anticonvulsant for controlling tonic-clonic and complex partial seizures. Although primidone was introduced in 1952, its mechanism of action is not easily determined due to the fact that it is metabolized to the active metabolites phenobarbital (1) and phenylethylmalonamide (PEMA) (10)[54] In fact, primidone was once considered to be an inactive compound and that its anticonvulsant activity was due to the metabolites (1) and (10).[54] However, it has been shown that the parent compound primidone exhibits anticonvulsant activity by raising the threshold for tonic-clonic seizures.[55] Due to the similarities in action between primidone, phenytoin and carbamazepine, primidone is believed to act on the neuronal membrane by modulating ion movements. Toxic side-effects, which are associated with primidone therapy, include drowsiness, weakness and dizziness. Tolerance usually develops on long-term use.

1.3.1.6.0.0. The Succinimides



(11) $R^1 = CH_3$, $R^2 = C_2H_5$, $R^3 = H$ Ethosuximide

(12) $R^1 = CH_3$, $R^2 = Ph$, $R^3 = CH_3$ Methsuximide

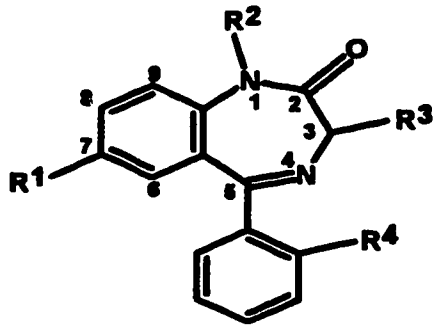
(13) $R^1 = H$, $R^2 = Ph$, $R^3 = CH_3$ Phensuximide

Ethosuximide (11), first introduced in 1960, is the drug of choice for treatment of absence seizures. Its effectiveness is clearly illustrated by a study[56] which showed that 95% of patients with absence seizures experienced a 50 - 100% reduction in seizure frequency. Furthermore, ethosuximide is well tolerated with the most common toxic side-effect being nausea. Although ethosuximide has been in use for the past three decades, little is known about its mechanism of action. Apart from the finding that it inhibits Na^+/K^+-ATPase,[57-58] there is no evidence to suggest an effect on membranes or interaction with neurotransmitters. Sohn and Ferrendelli[59] further showed that ethosuximide, unlike phenytoin and phenobarbital, does not inhibit influx of Ca^{2+} into synaptosomes. However, contradictory results were reported by Coulter *et al* [51,52,60] who reported that ethosuximide, like dimethadione, is effective in reducing the low threshold calcium current (T type calcium channels) in thalamic neurons. However, it is still too early to say whether abnormal T-calcium current is the cause of absence seizures, but this finding may help to shed light on its pathogenesis.

Apart from ethosuximide, methsuximide (12) and phensuximide (13) are also effective for controlling absence seizures. However, due to their lower efficacy, the latter drugs are used less frequently.

1.3.1.7.0.0. The Benzodiazepines

Diazepam (14) was introduced in 1968 under the trade name Valium®. Despite its sedative and antianxiety effect, diazepam is a drug of first choice for treatment of status epilepticus. Due to its high lipophilicity, diazepam is rapidly distributed to the brain to provide immediate seizure control. It has been reported[61] that two metabolites of diazepam, which include desmethyldiazepam (17) and oxazepam (18), are both active anticonvulsants although their concentrations are likely too low to exert a significant anticonvulsant effect. Chronic oral administration of diazepam leads to the development of tolerance.[62] Efficacy for seizure control is therefore lowered and a higher dosage regimen must be employed.



(**14**) $R^1 = Cl$, $R^2 = CH_3$, $R^3 = H$, $R^4 = H$ Diazepam

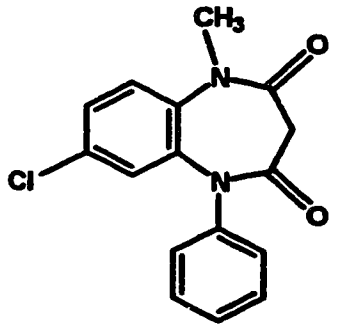
(**15**) $R^1 = NO_2$, $R^2 = H$, $R^3 = H$, $R^4 = Cl$ Clonazepam

(**16**) $R^1 = NO_2$, $R^2 = H$, $R^3 = H$, $R^4 = H$ Nitrazepam

(**17**) $R^1 = Cl$, $R^2 = H$, $R^3 = H$, $R^4 = H$ Desmethyldiazepam

(**18**) $R^1 = Cl$, $R^2 = H$, $R^3 = OH$, $R^4 = H$ Oxazepam

Clonazepam (**15**) and nitrazepam (**16**) also show a similar activity profile to that of diazepam, but they are more potent. Apart from controlling status epilepticus, the benzodiazepines clonazepam and nitrazepam are also effective for controlling absence, clonic and myoclonic seizures in infants.



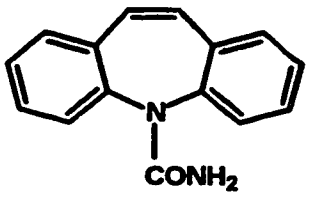
(**169**) Clobazam

Another analog of this class of A.E.D. is clobazam (**169**) which has a 1,5-benzodiazepine structure. Although it was marketed as an anxiolytic agent, clobazam (**169**) is effective for the treatment of myoclonic and generalized absence seizures.[232]

Like diazepam, the major limitation with clobazam (**169**) is the development of tolerance which can occur within weeks or months of administration.[233] Therefore, it is most beneficial for short-term treatment although it is often used as an add-on agent for the treatment of refractory epilepsies.

The anticonvulsant activity exhibited by benzodiazepines, like the barbiturates, is considered to be due to increased inhibition modulated by the GABA-chloride current.[63,64] Barbiturates and benzodiazepines both occupy allosteric sites associated with the $GABA_A$ receptor. Binding to the benzodiazepine site increases the affinity of GABA binding to its receptor and thus enhances the chloride current.

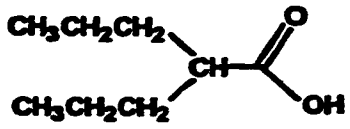
1.3.1.8.0.0. Carbamazepine



(**19**) Carbamazepine

Carbamazepine (**19**), which was approved in the U.S.A. in 1974, is a drug of choice for the treatment of complex partial and tonic-clonic seizures. Although carbamazepine has a similar therapeutic profile to phenytoin, its reduced toxicity has resulted in an increased use. Like phenytoin, carbamazepine is highly bound to plasma protein[65] (78 - 93%) which necessitates a higher dosage regimen. The most significant side-effects of carbamazepine are sedation, drowsiness and ataxia. The mechanism of action for carbamazepine, which is similar to that of phenytoin, involves the blocking of Na^+ channels which increases the threshold for burst-firing.[66]

1.3.1.9.0.0. Valproate



(20) Valproic acid

Valproic acid (20) was accidentally discovered to be an active anticonvulsant during the pharmacological testing of khellins where it was employed as a solvent. This resulted in the discovery that valproic acid is a potent agent against maximal electroshock and pentylenetetrazole induced seizures in rats and mice indicating its efficacy in protection against tonic-clonic and absence seizures. In 1978, valproic acid (Depakene®) was approved in the U.S.A. as an anticonvulsant for the treatment of a wide range of epilepsies including absence, partial, myoclonic and tonic-clonic seizures.

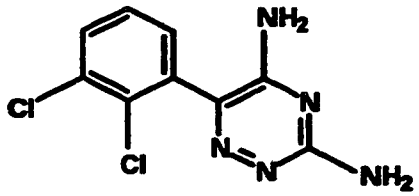
Gastrointestinal disturbances such as nausea and vomiting are the most common side-effects associated with valproate therapy. However, a greater concern is the rare occurrence of hepatotoxicity that occurs predominately in children on polytherapy with phenobarbital. The cause of this hepatotoxicity is not clearly understood, but it may be related to a reduction of the level of carnitine,[67] which is a cofactor responsible for mitochondrial oxidation of long chain fatty acids.

The mechanism of action for valproate has not been unambiguously elucidated although most evidence suggests an augmentation in brain GABA levels.[68] Valproic acid may inhibit GABA-transaminase,[68, 69] the enzyme responsible for the degradation of GABA. In addition, it may act as a GABA agonist by enhancing the activity of glutamic acid decarboxylase (GAD),[70] the enzyme responsible for synthesis of GABA by decarboxylation of glutamic acid. The hypothesis for GABA modulation by valproate is still controversial,[71] but this offers a most attractive model to explain its mechanism of action.

1.3.2.0.0.0. New and Potential Antiepileptic Drugs

Some 20 - 30% of epileptic individuals, as pointed out earlier, are refractory to currently available anticonvulant drugs.[7-9] Intensive efforts to develop new and more effective AEDs has taken place during the last decade. Three new drugs, lamotrigine, felbamate and gabapentin have recently been approved by the United States Food and Drug Administration (FDA), while others are undergoing advanced testing. In fact, some of these new drugs are already available in other countries. These new AEDs are now commonly used in monotherapy, or in combination with conventional anticonvulsants, to control various seizure types.

1.3.2.1.0.0. Lamotrigine

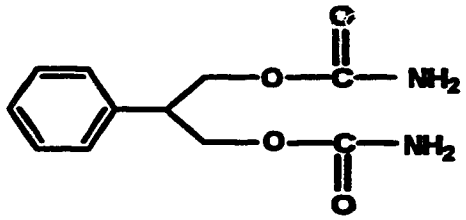


(21) Lamotrigine

Lamotrigine (21) is a novel anticonvulsant approved in the U.S.A. in March of 1993 as an add-on treatment for partial and secondary generalized seizures. Due to the similarity of its anticonvulsant profile to that of phenytoin and carbamazepine, lamotrigine is believed to have a similar mechanism of action[72, 73] where it blocks the voltage dependent Na^+ channels and stabilizes the inactivated channel state. This in turn would reduce excitatory neurotransmitter release such as glutamate and hence stablization of the neuronal membrane. It was reported[74] that administration of lamotrigine, in combination with valproate, increased the elimination half-life for lamotrigine, but that it was decreased by phenytoin and carbamazepine coadministration. Despite these drug interactions, lamotrigine is a non-toxic compound that is well

tolerated. The usual side-effects reported[75] for lamotrigine include dizziness, diplopia, somnolence, headache and ataxia.

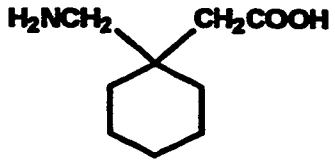
1.3.2.2.0.0. Felbamate



(22) Felbamate

Felbamate (22) is another new A.E.D. introduced which was found to be effective for controlling refractory partial seizures and Lennox-Gastaut Syndrome, that is defined as a severe epileptic syndrome marked by various types of generalized seizures found in early childhood.[76, 77] A remarkable feature of felbamate is its low toxicity. The lethal dosage (LD_{50}) in mice,[78] which is greater than 3000 mg/kg, is well above the effective dose for controlling seizures. A dosage up to 3600 mg/day can be administered, and in a combination with other AEDs, effectively suppresses partial onset seizures.[79] Serious side-effects associated with felbamate mono-therapy include gastrointestinal effects such as nausea, vomiting, and CNS side effects such as dizziness and insomnia.[79] The mechanism of action for felbamate has not been fully established. Felbamate blocks seizures induced by NMDA,[80] but it does not appear to bind to this receptor. It appears that felbamate's action may be on Na^+ channels as it was found to block repetitive burst firing. Although felbamate was approved for use in July, 1993, its use however was short-lived since a high rate of aplastic anemia associated with the administration of felbamate was reported.[234] As a result, the Food and Drug Administration in the U.S. and its manufacturer, the Carter-Wallace Inc. jointly recommended the withdrawal of felbamate use except in cases when it is absolutely necessary.

1.3.2.3.0.0. Gabapentin



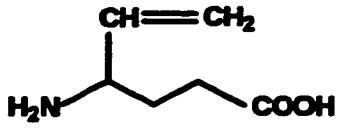
(23) Gabapentin

(24) GABA

Gabapentin (23) has a structure similar to that of the endogenous inhibitory neurotransmitter GABA (24). However, there is no evidence for its binding to the GABA receptor or its ability to modulate the metabolism of GABA.[81] Gabapentin does not interact with ion channels. It is therefore believed to have a novel mechanism of action different from all other conventional AEDs. Due to its structural similarity to L-leucine, a common amino acid found in the brain, it was postulated that gabapentin exerts its anticonvulsant activity by altering the concentration or metabolism of brain amino acids.[81]

A remarkable property of gabapentin is the fact that it is metabolically stable since it is eliminated unchanged by renal excretion. Accordingly, there are no drug interactions when gabapentin is coadministered with other AEDs. Gabapentin may be less toxic since a dose of up to 2400 mg/day [82] was devoid of any serious side-effects although somnolence, fatigue and dizziness have been observed in some individuals. In December of 1993, gabapentin was approved for clinical use in the U.S.A. as an add-on agent for the control of refractory partial seizures.

1.3.2.4.0.0. Vigabatrin



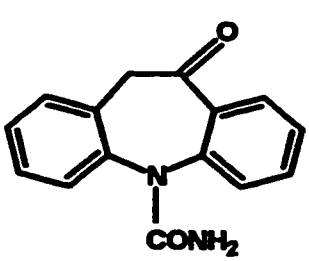
(25) Vigabatrin

Vigabatrin (γ-vinyl-GABA) (25) is a novel AED with a structure similar to that of GABA (24). Like gabapentin, vigabatrin was designed as a GABA mimic. Its mechanism of action involves the inhibition[83] of GABA-transaminase (GABA-T), the enzyme responsible for the degradation of GABA, by binding irreversibly to the active site of GABA-T. Consequently, GABA is spared from deactivation which results in an elevated brain GABA levels.

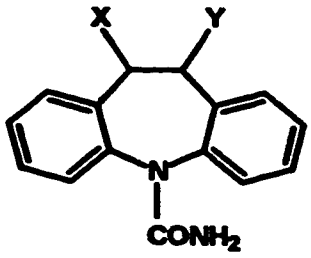
Clinically, vigabatrin is used as an add-on agent for the treatment of refractory partial, but not absence or myoclonic seizures. Its efficacy was illustrated by a study[84] which showed that ≥ 4 g/day oral dose given to refractory partial seizure patients was very effective. For example, 55% of individuals were found to have a ≥ 50% reduction in seizure frequency and that 15% were seizure free.

The development of vigabatrin as an AED was jeopardized by the discovery that micro-vacuolation in the CNS of rats and dogs occurred after chronic administration.[85] It was subsequently found that this phenomenon is species dependent and that it does not occur in humans. In fact, vigabatrin is well tolerated. Common side-effects include drowsiness, ataxia and fatigue. Vigabatrin has now achieved worldwide clinical acceptance.

1.3.2.5.0.0. Oxcarbazepine



(26) Oxcarbazepine



(27) X = H, Y = O

(28) X = Y = —O—

(29) X = Y = OH

Oxcarbazepine (26) is a keto analog of carbamazepine (19) which possesses a superior profile to that of carbamazepine since it provides a similar efficacy against partial and tonic-clonic seizures, but produces fewer side-effects. This reduced toxicity is due to the fact that the metabolism of oxcarbazepine differs from that of carbamazepine[86] which undergoes epoxidation to the epoxide (28). Further hydrolysis of the epoxide (28) yields the dihydroxy metabolite (29) which is inactive. Since oxcarbazepine does not possess a C(10) - C(11) C=C double bond, it undergoes metabolic reduction to form a monohydroxy derivative (27) which is an active metabolite with antiepileptic properties. The absence of an epoxide[87] intermediate may account for reduced toxicity relative to carbamazepine. Since metabolism of oxcarbazepine does not involve the hepatic microsomal enzyme cytochrome P450, drug-drug interactions which are common to most conventional AEDs do not pose a major problem. In a study[88] conducted on 947 epileptic patients, the most common adverse side-effects observed were dizziness, sedation and fatigue which indicates that oxcarbazepine is well tolerated. In fact, a dose as high as 3600 mg/day can be prescribed for some epileptic individuals without any serious side-effects.[89]

Although the use of oxcarbazepine is increasing, its mechanism of action is not fully understood. *In vitro* studies[90] employing oxcarbazepine, and its metabolite (27), resulted in suppression of sustained high frequency repetitive firing suggesting both

oxcarbazepine and its monohydroxy metabolite act by inhibition of sodium channels similar to phenytoin and carbamazepine. Oxcarbazepine has not been approved by the U.S.A. Food and Drug Administration, but clinical studies are in progress. However, oxcarbazepine is marketed in some European countries.

1.4.0.0.0.0. THE RELATIONSHIP BETWEEN CALCIUM AND SEIZURES

Although the mechanism of epileptogenesis is still not yet fully understood, increasing evidence points to a role for calcium in this dysfunction. It was reported[91] that the extracellular calcium concentration decreases in both electrical and pentylenetetrazole induced seizures. Further evidence was provided by Meldrum *et al*, [92-93] who reported that calcium deposits were found in various organelles of a hippocampal preparation and dendrites of rats after status epilepticus. Despite the fact that this enhanced Ca^{2+} entry is reversible,[93] there is speculation[94] that an overload of Ca^{2+} could cause cytotoxicity. This could lead to cell death in the hippocampus which is common in status epilepticus.

Although it is still controversial[95] as to whether excessive calcium influx is mediated through voltage-operated calcium channels or excitatory neurotransmitter mediated receptor-operated calcium channels, many calcium channel antagonists such as nifedipine,[96] nimodipine,[96-97] and flunarizine[98] are reported to control seizures. These results suggest a role for voltage-operated calcium channels as a potential pathway for calcium influx. Furthermore, it has been observed that the calcium channel agonist BAY K 8644, which stimulates calcium influx into cells, induces seizures in a dosage dependent manner.[99] All of these results implicate an important role for calcium in seizures.

1.4.1.0.0.0. The Homeostasis of Calcium in Cells

Calcium is no doubt the most important ion in many physiological processes. It can be regarded as a 'secondary messenger' comparable to that of cAMP.[100] Its primary role is to act as a cellular messenger by transmitting extracellular information into the interior of the cell. Of the many important roles for Ca^{2+}, the most remarkable is its involvement in the excitation-contraction coupling of the heart, smooth and voluntary muscle. The excitation-secretion coupling of various enzymes, hormones and neurotransmitters are also important.

These important roles for Ca^{2+} dictate a strict control of its movement and storage. The concentration of Ca^{2+} inside a living cell is about 10^{-7} M, whereas the extracellular concentration is usually about 10^{-3} M. This large concentration gradient is partly maintained by the lipid bilayer on the plasma membrane. Due to the lipophilic nature of the plasma membrane, ions are not able to enter cells except through certain channels. Ca^{2+} homeostasis is also maintained by sequestration in intracellular stores such as sarcoplasmic reticulum, mitochondria and the ubiquitous calcium binding protein calmodulin. Calcium pumps on the plasma membrane can help to extrude Ca^{2+} against the concentration gradient by hydrolysis of ATP. These homeostasis mechanisms are important, as uncontrolled influx of Ca^{2+}, such as that produced by toxins and certain disease states can kill the cell.[101]

1.4.2.0.0.0. Types of Calcium Channels

There are two types of calcium channels. Receptor-operated calcium channels (ROC) respond by ligand-binding to extracellular receptors, whereas voltage-operated calcium channels (VOC) are modulated by an electrical potential across the plasma membrane. The VOC has been studied more extensively. The invention of the whole-cell patch-clamp technique allows one to monitor the kinetics of a single channel. The discovery of compounds which modulate (agonists and antagonists) calcium channels, resulted in the characterization of four types of VOCs called T, L, N and P. These

calcium channels have been classified according to their electrophysiological and pharmacological responses.[102, 103]

T-type channels are located mainly in excitable cells such as the cardiac sinoatrial (SA) node, neurons and endocrine cells. The T-channel has a low threshold and hence it is opened by a low depolarization. As its name implies, it has a transient (T) open time and is rapidly deactivated. Pharmacologically, the T-channel is sensitive to octanol and amiloride.

The L-type channels can be found in most excitable cells particularly in the cardiovascular system, neurons and endocrine cells. The L-channel is the only channel that is sensitive to synthetic calcium channel antagonists/agonists. Electrophysiologically, it has a high threshold for activation. As its name implies, the L-channel opening is long lasting (L) and it is slow to deactivate.

N-type channels are present mainly in neurons and are considered to be responsible for neurotransmitter release. Like the L-type, N-channels have a high threshold for activation, but a moderate rate of deactivation. N-channels are insensitive to calcium channel antagonists, but are blocked by ω-conotoxin.

P-type calcium channels are present in cerebellar purkinje cells and are also considered to be responsible for neurotransmitter release. P-channels have a moderate threshold for activation, but are deactivated rapidly. Pharmacologically, P-channels can be blocked by funnel web spider toxin.

1.4.3.0.0.0. Structure of the L-type Calcium Channel.

Most studies involving the L-type Ca-channel use skeletal muscle as the source tissue due to its high calcium channel density. The discovery of calcium channel antagonists in the late 1960's facilitated elucidation of the molecular nature of L-type channels. Using different types of [^{3}H]-calcium channel antagonists as molecular probes and electrophoresis techniques, it was possible to identify a single polypeptide of 145 - 170 kDa as the calcium channel antagonist receptor.[104] Purification of the calcium channel containing protein gave in addition to the receptor protein (α_1-subunit), two other polypeptides which were later named β and γ subunits, respectively. Further

investigations[105] by cleavage of disulphide bonds revealed two other subunits α_2 and δ indicating that the channel is an oligomeric complex of 5 subunits, α_1, α_2, β, γ and δ as shown in Figure 2.

The α_1-subunit, which contains the binding sites for the three classes of calcium channel antagonists (dihydropyridine, phenylalkylamine and benzothiazepine), also plays an important role in controlling gating of the channel. Tanabe *et al*[106] determined the amino-acid sequence of the α_1-subunit by cloning and sequencing the cDNA and its complimentary mRNA for the rabbit skeletal calcium channel. A putative structure containing 4 domains each with 6 α-helical transmembrane segments S1 - S6 was proposed. (see Figure 2) These 4 domains with their transmembrane segments form the calcium channel. In addition, the S4 unit of each domain was found to have positive-charged amino acid residues such as arginine and lysine in every third position in the sequence which were believed to act as voltage sensors for the channel. Amazingly, this arrangement was found to be highly homologous to the α-unit of the sodium channel indicating a similar control mechanism for these two types of channels.

Further work[107, 108] revealed the amino acid sequence for the α_2 and β subunits, that lack homology to other proteins. Starting in the late eighties, there has been dramatic progress due to the molecular cloning of L-type channels. In addition, sequencing calcium channel receptor protein from sources other than skeletal muscle has provided structural information for dihydropyridine insensitive channels.[103] These, and future studies, will provide information pertaining to the molecular nature and binding interactions to calcium channels.

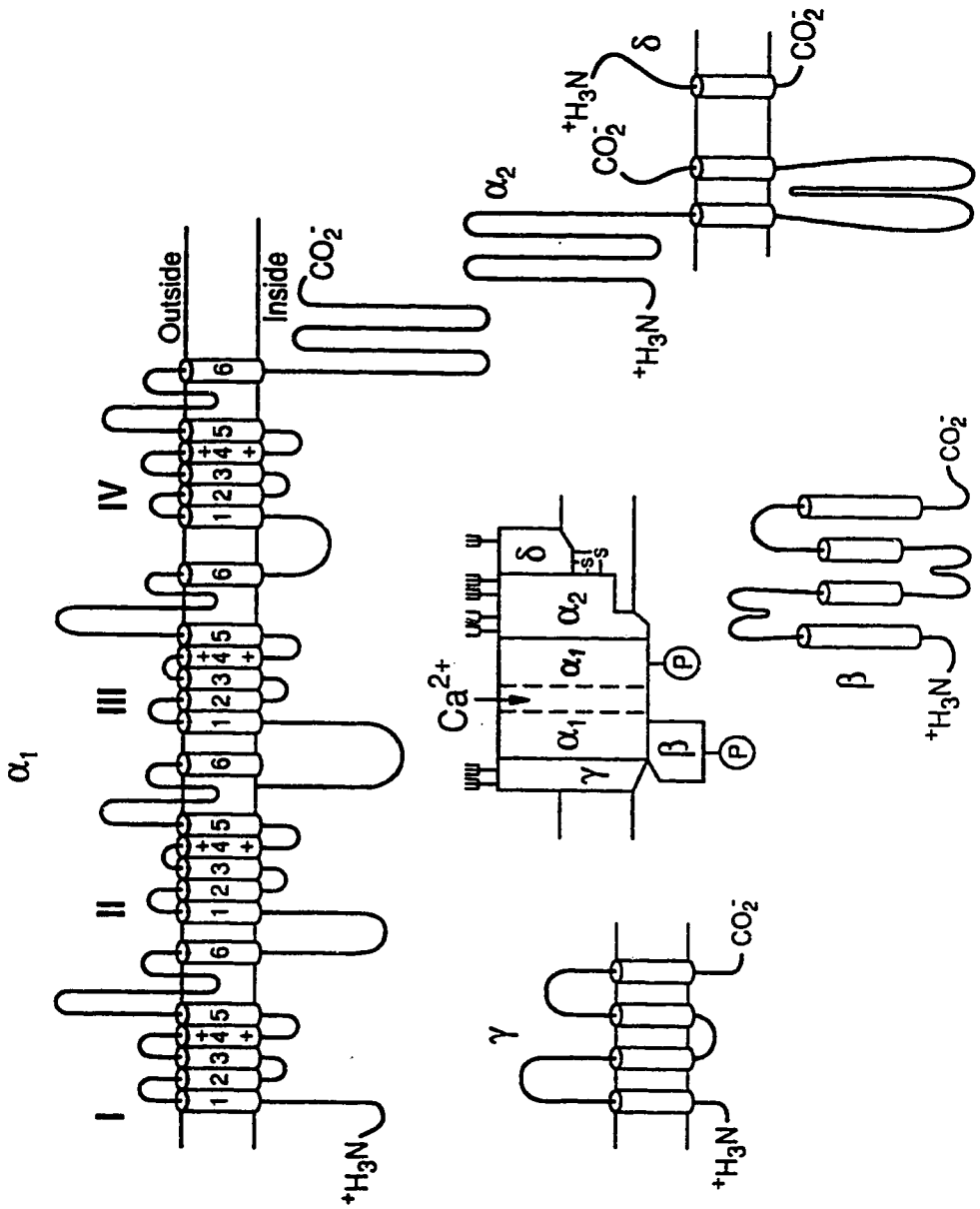


Figure 2. The structure of the L-type Ca-channel and its subunits. The α_1-subunit forms the channel while phosphorylating sites, as indicated by P , are present in the α_1 and β-subunits. Reprinted with permission from Birkhaüser Verlag, Basel, Switzerland. Taken from Rampe; Triggle. New Synthetic Ligands for L-type Voltage-gated Calcium Channels. In *Progress in Drug Research*, **1993**, *40*, p 197.

1.4.4.0.0.0. Regulation of the Calcium Channels

Ion channels are highly selective towards ions. It is incorrect to assume that permeation of ions is passive and that ion discrimination is entirely due to properties of the ion alone. Hagiwara and Takahashi[109] discovered that the calcium current increases when extracellular calcium is at a low concentration. The observation that current saturation occurs at high Ca^{2+} concentrations indicates that an interaction between the ion and the channel does occur. Hess and Tsien[110] proposed a two ion binding site hypothesis where occupation of a neighbouring site by Ca^{2+} could induce an electrostatic repulsion of the other Ca^{2+} ion during generation of an ionic current and the prevention of ion saturation on the channel. Other than these data, the detailed mechanism for permeation of ions through channels is still not clear.

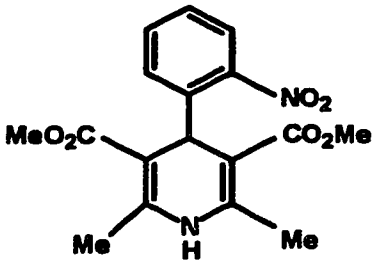
Although VOCs are activated by depolarization of the membrane potential, they are also modulated by receptor binding. As illustrated in Figure 2, there are sites on the α and β subunits which can be phosphorylated by cAMP-dependent protein kinase. Receptor binding via adenylate cyclase and cAMP could also effect phosphorylation of calcium channel proteins, and thus modulate channel activity.

1.5.0.0.0.0. CALCIUM CHANNEL ANTAGONISTS

The term 'calcium channel antagonists' (CCAs), also called 'calcium blockers' or 'calcium antagonists' is defined[111] as "substances that specifically and predominately inhibit the slow inward current of calcium ions. Other effects such as inhibition of the fast inward sodium current, should play no role." Although the first CCA, cinnarizine, was introduced in the 1950's, its mechanism of action[112] which involved inhibition of the inward calcium current was not known until 1968. Later, Fleckenstein[113] was able to show the calcium antagonist effect exhibited by verapamil, which was described as a 'calcium antagonist'. During the 1970's and 1980's there was a rapid development of new CCAs. Although all CCAs are similar with respect to their ability to inhibit calcium current influx via the L-type calcium channel, they differ markedly in terms of their tissue

selectivity, duration of action and possibly the pharmacological application. Hence, clinical applications may be vastly different. In 1985, a committee appointed by the World Health Organization (WHO) devised a proposal[114] for the clinical and pharmacological classification of CCAs.

Despite the fact that many different compounds exhibit calcium antagonist activity, there are 4 principle groups of compounds that have been classified as CCAs.



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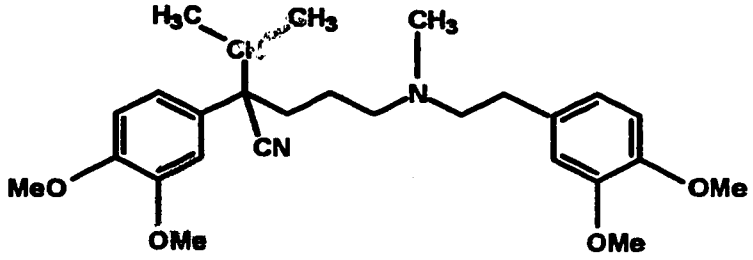

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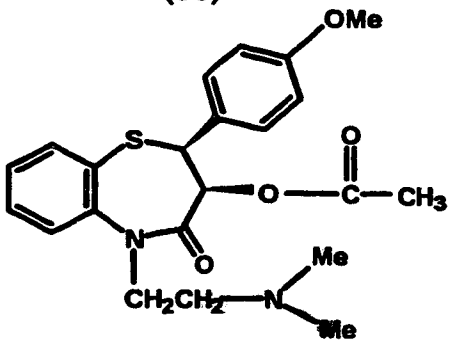

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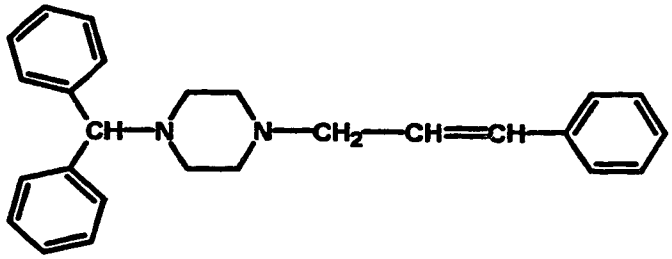


(33)

The first group is the 1,4-dihydropyridine class, as represented by nifedipine (30), which was found to be a potent vasodilator with selectivity for the peripheral vasculature. The second group is the phenylalkylamine class for which verapamil (31) has been selected as an example. Verapamil is more selective for cardiac tissue. Diltiazem (32) is another class of compounds that possess a benzothiazepine structure. Like verapamil, diltiazem is selective for cardiac tissue. The fourth class of compounds is represented by cinnarizine (33) which possess a benzopiperazine structure.

1.5.1.0.0.0. Clinical Applications of Calcium Channel Antagonists

CCAs are rather unique due to their wide range of potential therapeutic applications. In fact, many compounds[115] which are not considered to be CCAs bear some relationship to calcium inhibition as part of their mechanism of action. This is not surprising in view of the important role that calcium plays in many physiological processes.

Since calcium is essential for the excitation-contraction coupling process in cardiac and vascular beds, CCAs are used primarily for the treatment of cardiovascular disease.[116] Verapamil and diltiazem, which are cardio-selective, are effective for the treatment of arrhythmias, a disorder influenced by cardiac pacemaker cells and atrial-ventricular (A-V) conduction. Abnormal rhythms can be suppressed by inhibiting the slow calcium current. Dihydropyridines, such as nifedipine which are more vasoselective, are good vasodilators. In addition to its action on the coronary arteries, nifedipine is also effective in dilating arterioles thereby reducing vascular resistance. Recent evidence[117] suggests that CCAs are also effective inhibitors of atherosclerosis. Thus, the risk of coronary artery stenosis and myocardial ischemia can be reduced.

Besides their cardiovascular applications, CCAs are also effective for the treatment of cerebral vascular disorders. CCAs with high lipophilicity are able to cross the blood brain barrier (BBB) by passive diffusion to provide cerebral selectivity. The lipophilic 1,4-dihydropyridine analogs nimodipine[118] and nicardipine[119] have been evaluated for their ability to treat subarachnoid haemorrhage. These latter compounds provided protection from cerebral infarction or vasospasm which is common in stroke patients. Migraine, can also be considered as a common cerebral disorder. While the pathogenesis of migraine has not been fully resolved, it is due, at least in part, to excessive cerebral vasodilation following cerebral hypoxia initially caused by vasoconstriction.[120] A review by Spierings[121] states that CCAs such as flunarizine, nifedipine, nimodipine and verapamil are effective for the treatment of migraine. Among these, flunarizine is the most promising drug for reducing headache frequency for most patients (39 - 66%). Flunarizine is marketed in Canada as an antimigraine agent.

Another interesting application for CCAs is their potential for use as novel neuroleptics. Although this field of study is new, there has been considerable interest in their use to alter mood disorders. Höschl[122] in his review, indicated that verapamil is comparable in efficacy to lithium for the treatment of manic depression and suggested its possible use as a prophylactic drug to prevent depression.

The use of CCAs as antineoplastic agents is a new concept which has attracted much attention.[123] There are two theories for the antineoplastic activity of CCAs. The first theory is based on the hypothesis that CCAs act as calmodulin antagonists. Since calmodulin and calcium are essential for DNA synthesis, inhibition of the former substrate would suppress the proliferation of tumour cells. Some CCAs such as prenylamine[124] and niguldipine[125] have been reported to be effective calmodulin antagonists that suppress the growth of tumour cells in different cell lines. A second theory is based on the hypothesis that CCAs enhance the cytotoxicity of antitumour agents in multidrug resistance tumour cells. This latter effect is achieved by binding of the CCA molecule to P-glycoprotein which is responsible for the efflux of drugs from the tumour cell. Verapamil and dihydropyridines such as niguldipine[126] were found to enhance the accumulation of anticancer drugs in tumour cells. Modulation of P-glycoprotein by CCAs was found to be non-stereoselective. This is highly beneficial since binding of CCAs to the calcium channel receptor is usually stereoselective. Therefore, selecting the less potent enantiomer with respect to CCA activity as the antineoplastic agent would minimize the potential cardiovascular side effect.

The above indications are just a few of the many potential applications of CCAs that are now used clinically, or that may be developed in the future. Other applications under investigation include the treatment of asthma,[127, 128] renal protection[129, 130] and prevention of hepatotoxicity.[131]

1.5.2.0.0.0. Mechanism of Calcium Channel Modulations

Calcium channels are regarded as pharmacological receptors. Radioligand binding studies have shown that the three classes of CCAs, 1,4-dihydropyridine,

benzothiazepine and phenylalkylamine bind to three different sites on the α_1-subunit of the L-type calcium channel. These three CCA binding sites are allosterically related (see Figure 3a).[102] In addition to modulating the L-type calcium channel, binding to one site alters the affinity of the two other binding sites with respect to binding to their ligands.

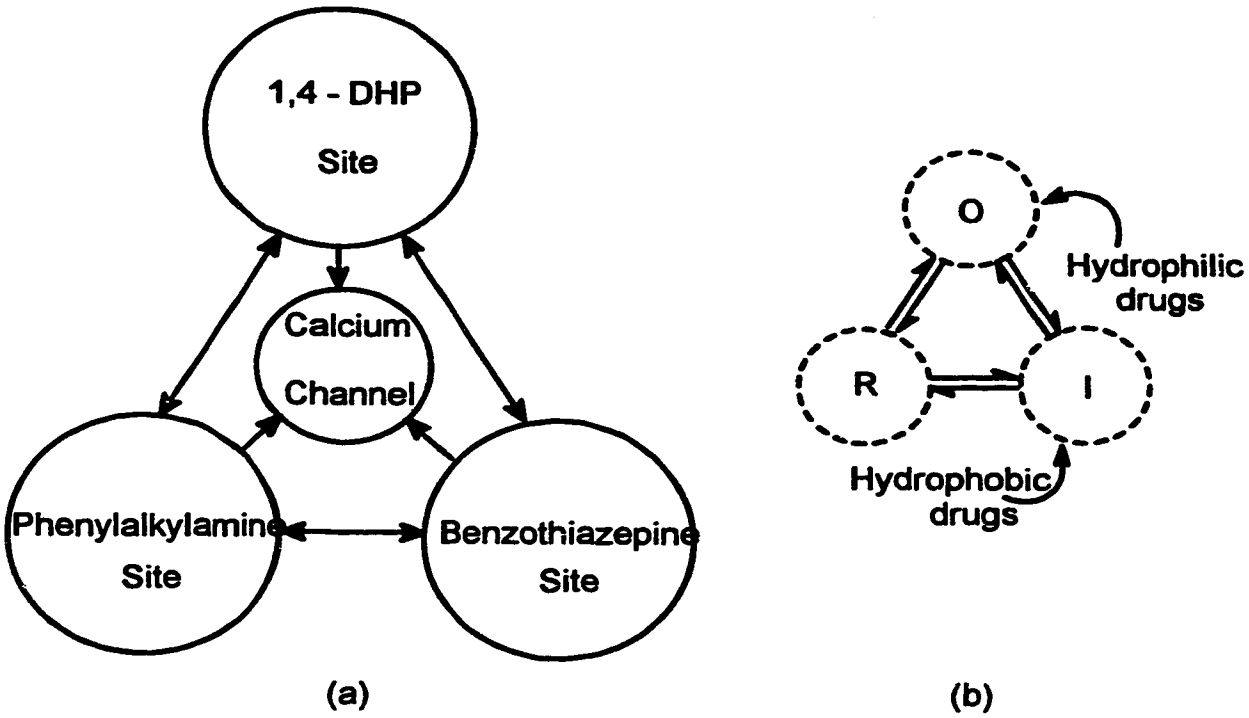


Figure 3 (a) Diagram showing the three allosteric sites of the calcium channel. (b) Diagram showing the interconversion of the three states of calcium channel. O = open state, R = resting state and I = inactivated state. Hydrophilic drugs bind to the open state an hydrophobic drugs bind to the inactivated state.

Regulation of the calcium channel involves at least 3 states for the channel (see Figure 3b). Upon depolarization, the channel is in an open-state (O) during which permeation of Ca^{2+} ions occurs. In order to limit Ca^{2+} ion flow, the channel converts to an inactivated-state (I) where the channel is closed and it cannot be activated by depolarization. After a period of time, when the channel is in the resting-state (R), it is once again amenable to activation upon depolarization.

The nature of drug binding to the channel receptor and the subsequent action is best explained by the Modulated Receptor Hypothesis,[132] which in its simplest form, states that the affinity of the CCA drug is affected by the channel state, whereas channel gating is modulated by the bound drug. It is clear from this hypothesis that binding of the CCA to the channel receptor is a state-dependent process. Hydrophilic drugs such as verapamil and diltiazem bind preferentially to the open-state which then undergoes transformation to the inactivated-state, whereas hydrophobic CCAs such as nifedipine bind to the inactivated-state (see Figure 3b). This hypothesis also suggests that once the CCA drug is bound, it stabilizes the inactivated-state of the channel. Thus, CCAs are thought to stabilize the inactivated-state, or to delay the recovery from inactivation. This hypothesis also offers an explanation for the tissue-selectivity of CCAs that may be due in part to the state-dependent interaction of the CCAs since the equilibrium among the three states is tissue-dependent.

1.6.0.0.0.0. THE CHEMISTRY OF 1,4-DIHYDROPYRIDINES

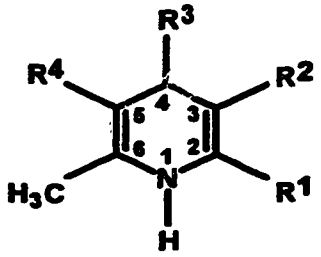
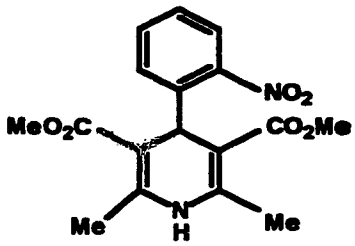


Figure 4. Substituent positions on the 1,4-dihydropyridine ring system that have been modified to acquire structure-activity correlations.

1,4-Dihydropyridines (DHPs) represent the most extensively studied class of CCAs. Extensive structure-activity correlations have been acquired by modifying substituents at the R^1, R^2, R^3 and R^4 positions on the 1,4-dihydropyridine moiety as shown in Figure 4. Nifedipine (30) (Adalat®), the first 1,4-DHP CCA drug, was introduced in 1975 for the treatment of coronary disease.

The clinical success of nifedipine provided a strong stimulus to develop new DHP derivatives. Many 'second-generation' DHPs have since been developed that differ considerably in their potency, tissue-selectivity and duration of action.[133] Nisoldipine (34) and nitrendipine (35) are nifedipine-like analogs that exhibit potent coronary and peripheral artery vasodilating activity with longer durations of action. Felodipine (39) is



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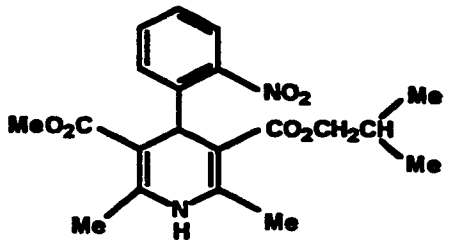

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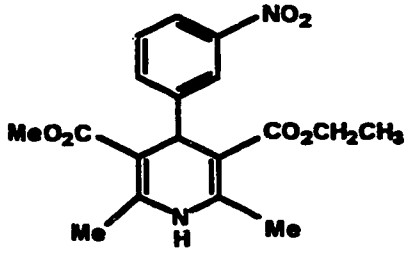

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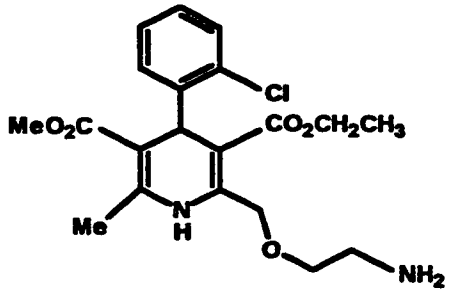

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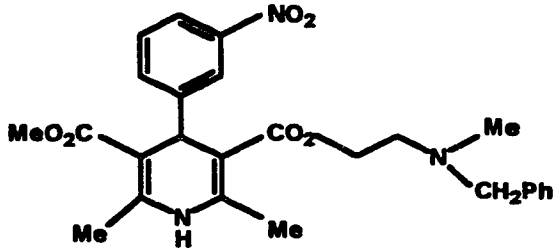

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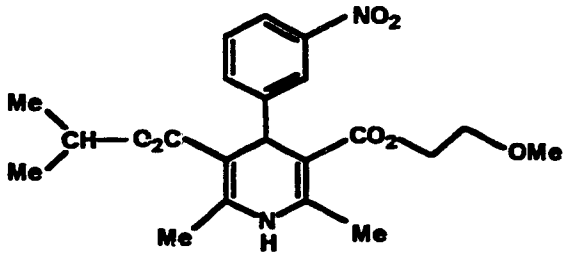

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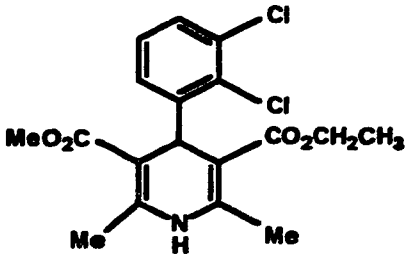


(39)

8-fold more vasoselective than nifedipine and 120-fold more selective than verapamil for vascular versus cardiac tissue.[102] Felodipine is therefore an effective hypotensive agent that is devoid of a negative inotropic effect. Amlodipine (36),[134] which has an aminoethoxymethyl C-2 substituent is a potent antihypertensive agent with a long duration of action and a plasma half-life of 30 hours. Nicardipine (37), a peripheral

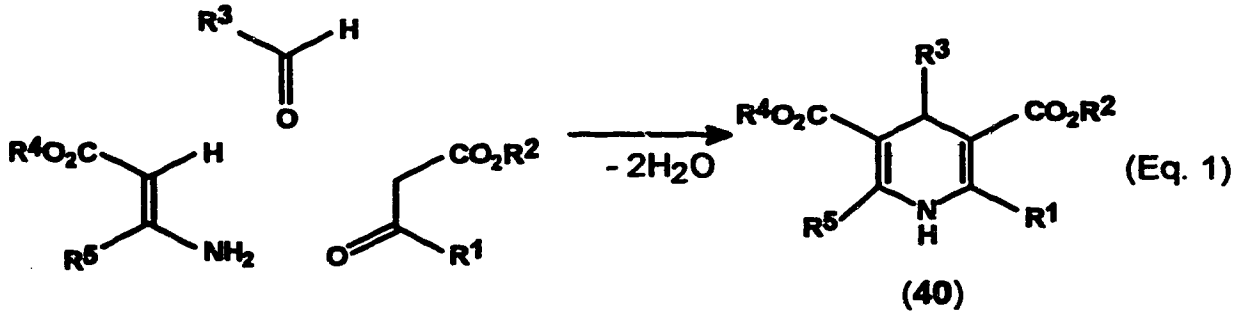
vasodilator, is also a cerebral vasodilator. Nimodipine (**38**), which is highly lipophilic, is a cerebral-selective vasodilator that increases cerebral blood flow that is beneficial for the treatment of cerebral disorders.

Nifedipine is an achiral symmetrical DHP since the ester groups at C-3 and C-5 are the same. In contrast, the DHPs (**34** - **39**) which are non-symmetrical and possess a chiral carbon at the C-4 position exist as a racemic mixture of two enantiomers. The CCA activity exhibited by the two enantiomers is usually different due to stereoselective binding to the chiral calcium channel receptor. Certain chiral DHPs, to be discussed later, exhibit opposite pharmacological activity for the two enantiomers (see 1.7.3.0.0.0.). A review[135] of the various methods for resolution of racemic DHPs has been published.

1.6.1.0.0.0. Synthesis of 1,4-Dihydropyridines

1.6.1.1.0.0. Hantzsch Synthesis

The primary reason for the large number of DHPs investigated can be attributed to the synthetic method devised by Hantzsch[136] more than a century ago. The classical "Hantzsch synthesis" can be modified in a number of ways depending on the starting materials available. For a detailed account of this synthesis, readers are directed to a review by Kuthan and Kurfürst.[137]

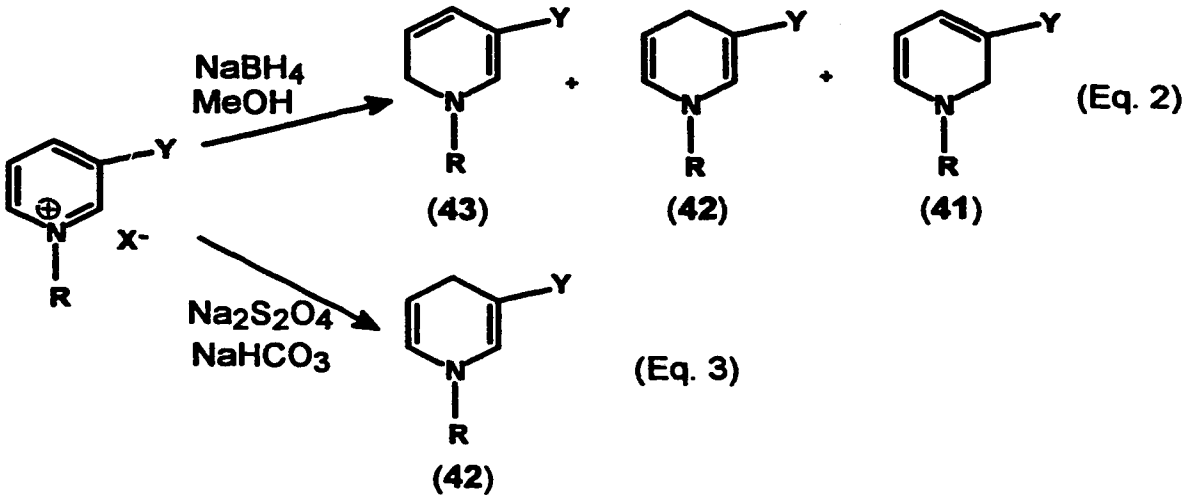


(40) (Eq. 1)

A simplified Hantsch synthesis is illustrated by (Eq 1) where an aldehyde is condensed with an enamine ester and a β-ketoester to form the DHP (**40**).

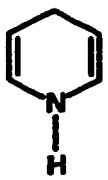
1.6.1.2.0.0. Reduction of a Pyridinium Salt

1,3-Disubstituted pyridinium salts are readily reduced with sodium borohydride to yield a mixture of 1,6- (**43**), 1,4- (**42**) and 1,2-dihydropyridine products (**41**) (Eq. 2). The ratio of the dihydropyridyl isomers formed is dependent on the reaction conditions

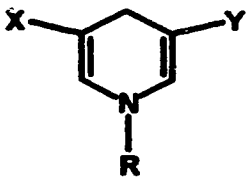


(solvent, temperature) and the nature of substituents R, Y. For example,[138] when R = Me, Y = $CONH_2$, X = $MeSO_4^-$, a mixture of the 1,4- and 1,6- isomers (**42** and **43**) are formed while R = Ph, Y = $CONH_2$, X = Cl^- can yield a mixture of 3 isomers (**43**, **42**, and **41**). However, the use of sodium dithionite[139, 140] as the reducing agent in an alkaline medium yields the 1,4-dihydropyridine (**42**) as the sole product (Eq. 3). This reduction method is therefore best used for the synthesis of nicotinamide adenosine dinucleotide (NAD) mimics.[137, 141]

1.6.2.0.0.0. Reactions of 1,4-Dihydropyridines



(**44**)

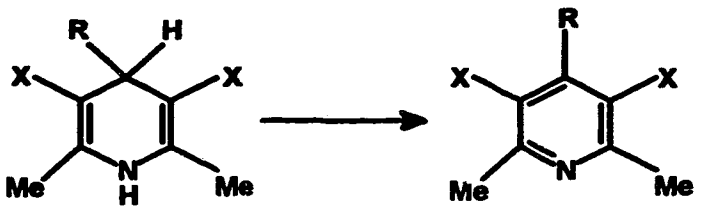


(**45**)

Although the 1,4-DHP (44) is thermodynamically more stable than the corresponding 1,2-dihydropyridine isomer, (44) is very unstable at room temperature.[142] Electron-withdrawing substituents X and Y at position 3 and/or 5 such as $-NO_2$, -CN, -COR, and $-CO_2R$ stabilize the 1,4-DHP ring system (45) due to conjugation with the enamine moiety.[137]

1.6.2.1.0.0. Aromatization

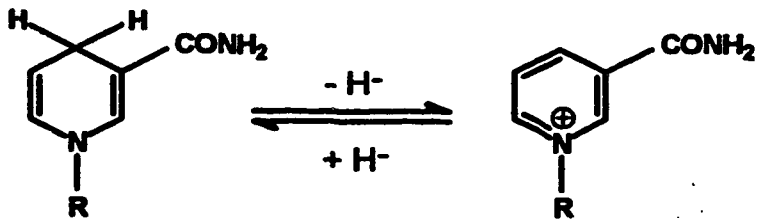
The most common reaction of the 1,4-dihydropyridine ring system involves dehydrogenation at the 1- and 4-positions to yield an aromatic pyridine.[143] (Eq 4).



(Eq. 4)

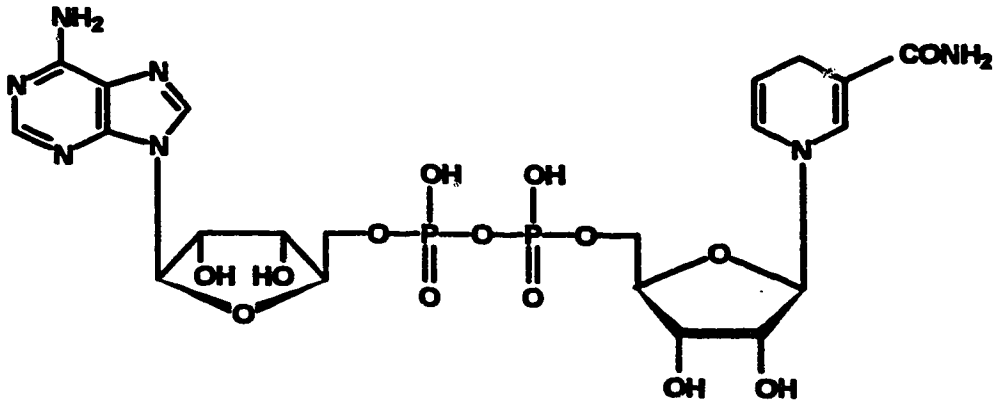
This oxidation reaction can be effected using oxidizing agents[140] such as nitric acid, hydrogen peroxide, chloranil or air. The ease of oxidation[144] is dependent on the nature of the R- and X- substituents where the relative ease of oxidation is R = H > Me > Ph and X = H > COR > CO_2R > COPh > CN. Hence, DHPs with unsubstituted 3- and 5-positions, which are less stable, are easily aromatized.

Perhaps the most interesting oxidation of a DHP is that involving 1,4-dihydronicotinamide (46) which undergoes oxidation to the pyridinium salt (47) (Eq.5).



(46) (47)

(Eq. 5)

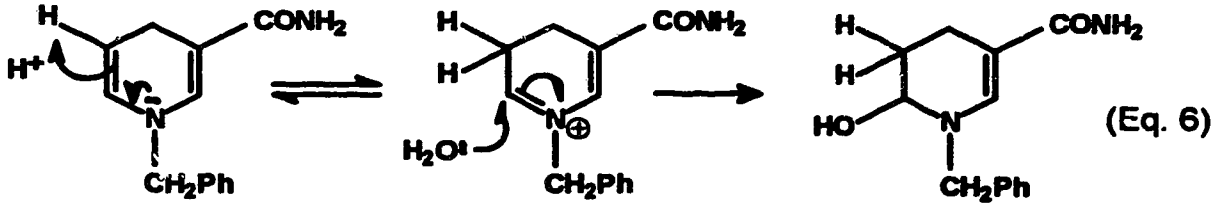


(48)

The importance of this reaction is illustrated by the biochemical redox system for the endogenous coenzyme nicotinamide adenosine dinucleotide (NADH) (48) and the pyridinium salt NAD. There have been many mimetic reactions[137] carried out to study the mode of proton transfer. However, the mechanism is still controversial as to whether it involves a free radical process[145-147] (single electron transfer) or a hydride transfer.[148-149]

1.6.2.2.0.0. Acid-Base Properties

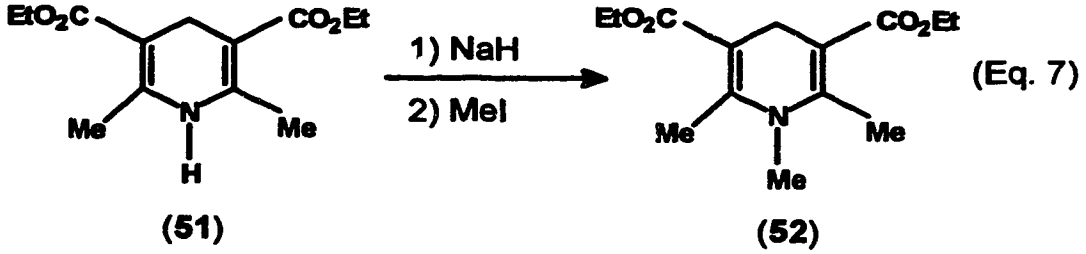
Although 1,4-DHPs possess a nitrogen atom with an electron lone-pair, 1,4-DHPs are not basic.[140] Protonation or alkylation to form quaternary salts is not easily effected. However, facile hydration occurs at the C-6 position of some 1,3-disubstituted 1,4-DHPs, such as (49), which yields the 1,4,5,6-tetrahydropyridine[150] (50) (Eq. 6).



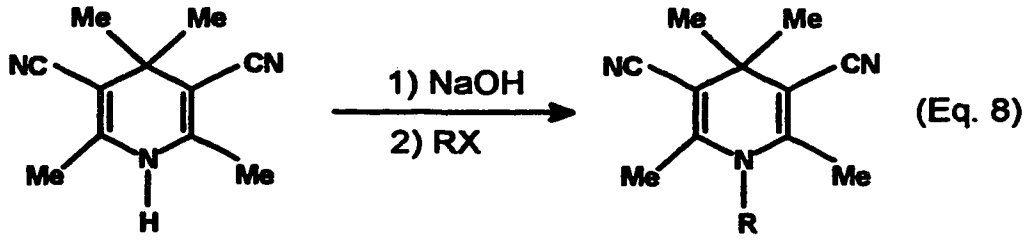
(Eq. 6)

(49) (50)

The acidic property of 1,4-DHPs is evident since deprotonation using strong bases such as sodium hydride, organometallic reagents such as Grignard reagents, or phenyllithium is facile. The resulting anion is a strong nucleophile that reacts with alkylating reagents to yield N-alkylated-1,4-DHPs. An example[151] is shown by (Eq. 7) where the DHP (51) is methylated at the 1-position to afford the N-methyl-DHP (52). More acidic DHPs such as 3,5-dicyano-2,4,4,6-tetramethyl-1,4-dihydropyridine (53)[152] can be N-alkylated using aqueous NaOH in the presence of a phase-transfer catalyst (Eq 8).



(51) (52) (Eq. 7)

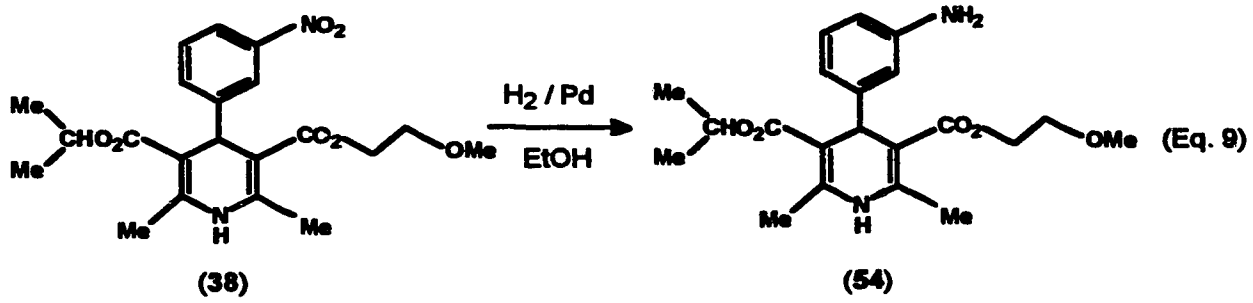


(53) (Eq. 8)

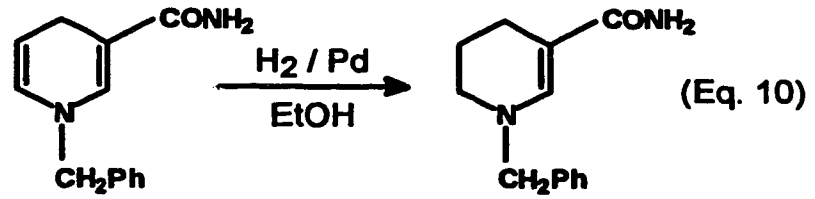
1.6.2.3.0.0. Reduction of 1,4-Dihydropyridines

Hantzsch dihydropyridines (1,4-DHPs with electron withdrawing substituents at the 3-and 5-positions) cannot be reduced by hydrogenation. This is due to conjugation of the 1,4-DHP enamine moiety to the C-3 and C-5 electron-withdrawing groups.[137] Thus, hydrogenation of nimodipine (38) with H_2 in the presence of Pd catalyst, resulted in a specific reduction of the nitro group to afford the amine (54) leaving the dihydropyridine moiety intact (see 3.1.9.0.0.0.) (Eq. 9).

However, in the case of the 1,3-disubstituted-DHP (55), partial hydrogenation of the unconjugated olefinic bond to afford the 1,4,5,6- tetrahydropyridine (56) does occur[153] (Eq. 10).



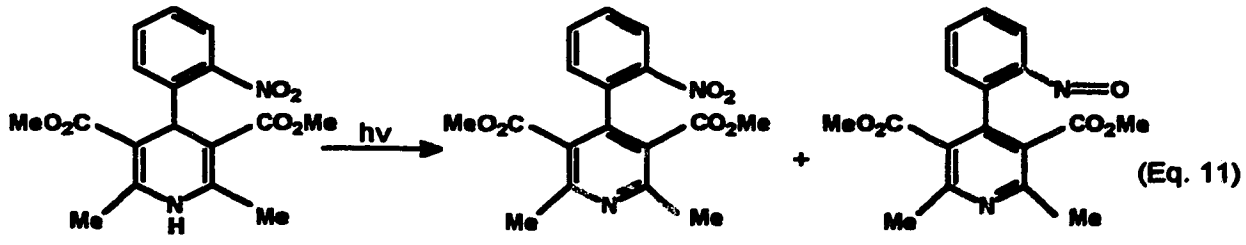
(38) (54) (Eq. 9)



(55) (56) (Eq. 10)

1.6.2.4.0.0. Photochemical Reactions

Although 1,4-DHPs are generally considered to be light-sensitive,[133] studies describing photochemical reactions are scarce. Nifedipine (30) was reported[154] to undergo aromatization during photolysis to yield a 2-nitrophenyl pyridine (57) and a 2-nitrosophenyl derivative (58) (Eq 11).



(30) (57) (58) (Eq. 11)

1.7.0.0.0.0. STRUCTURE -ACTIVITY RELATIONSHIPS (SARs) FOR 1,4-DIHYDROPYRIDINE CALCIUM CHANNEL MODULATORS

Loev *et al* [155] determined the hypotensive activity exhibited by 79 1,4-DHPs and some aromatic pyridine analogs which provided the first qualitative structure-activity correlations for 1,4-DHP CCAs. The 1,4-dihydropyridine ring was essential since the aromatized pyridine or reduced piperidine[156] analogs were inactive. The N-H was also essential since N-substituted analogs exhibited decreased CCA activity. An aryl or heteroaryl substituent at C-4 increased activity. The relative activity order for substituents on the C-4 aryl ring was *ortho* > *meta* >> *para*. The 3,5-positions are preferably esters substituents since other electron-withdrawing groups (CO, CN) reduced activity. Introduction of a C-3 nitro substituent often confers calcium channel agonist activity (see 1.7.3.0.0.0). The 2- and 6-positions are usually occupied by methyl groups. However, introduction of larger groups such as a 2-aminoethoxymethyl substituent as in amlodipine (**36**), or a CN group, at the C-2 and C-6 positions are allowed.

1.7.1.0.0.0. Conformation of 1,4-Dihydropyridines

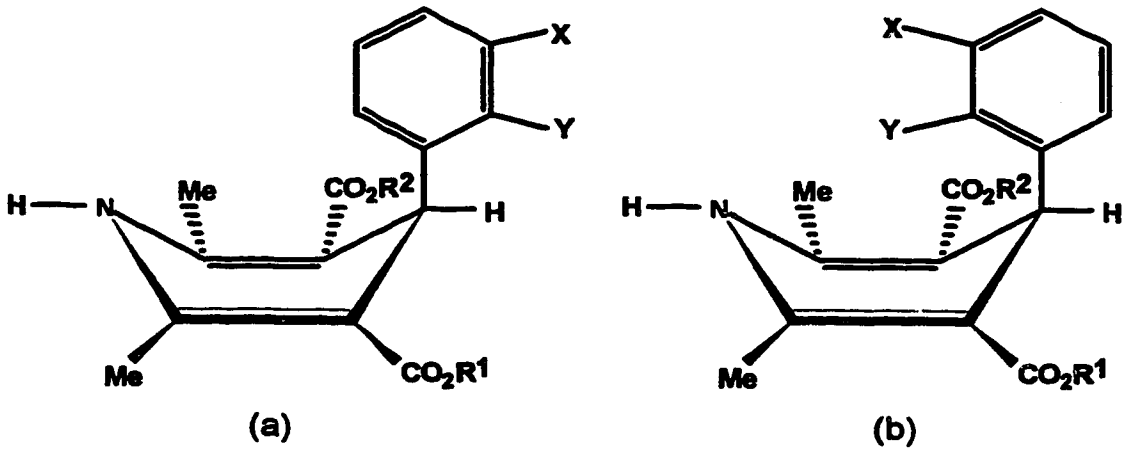


Figure 5. The conformation of Hantzsch dihydropyridines with a C-4 aryl substituent. (a) and (b) represent two possible rotamers with respect to the phenyl ring orientation.

Many studies have been carried out to determine the conformation of 1,4-DHPs. X-ray diffraction on crystals[157-159] and various quantum chemical calculations[160-163] indicate a relatively flat 1,4-dihydropyridine boat-shaped structure, although the degree of distortion of the boat (flatness) is dependent upon the positions of substituents in the C-4 phenyl ring and at the 1,4-DHP C-3 and C-5 positions (Figure 5a).

The phenyl ring at the C-4 position is oriented pseudoaxial to the plane of the 1,4-DHP ring. Any attempt which forces the C-4 phenyl substituent to be equatorial such as that in 4,4-disubstituted DHPs abolishes CCA activity.[164] Although free rotation about the C-(4)-phenyl bond could result in several possible rotamers, the phenyl ring is orthogonal to, or it bisects the plane of, the dihydropyridine ring. For *ortho-* and *meta-*substituented phenyl substituents, there are thus two possible rotamers. The X- and -Y substituents can be on the same side as the C-4 hydrogen (synperiplanar, sp) (Figure 5a) or on the opposite side (antiperiplanar, ap) (Figure 5b). Although X-ray studies indicate a sp orientation for 1,4-DHP CCAs, this may not accurately reflect the conformation in solution, or the conformation which binds to the DHP-receptor site. Nuclear Overhauser Enhancement (NOE) studies[165-166] indicated that the sp-rotamer predominates in solution. A correlation between vasorelaxant activity and receptor binding affinity support the sp-rotamer.

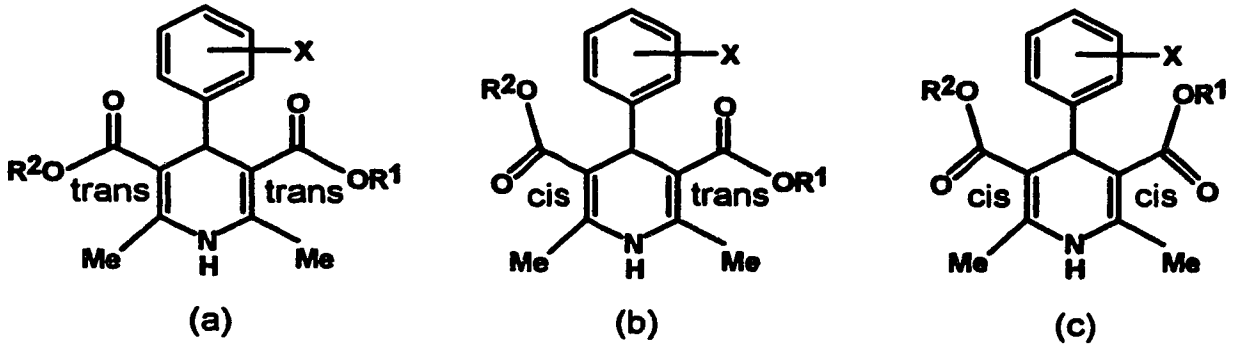
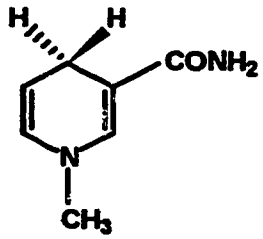


Figure 6. Three possible conformations of the Hantzsch dihydropyridine caused by rotating the ester substituents at the 3,5-positions.

The two esters groups at the C-3 and C-5 positions are equatorial and oriented in the same plane as the C=C bond of the dihydropyridine ring. Free rotation about the bond between carbonyl group (C=O) and C-3 or C-5 carbon atom makes two rotamers possible (on the same side (*cis*) or opposite (*trans*) the double bond on the dihydropyridine ring). When two ester groups are present at C-3 and C-5, three conformations are possible as shown in Figure 6 having *trans/trans* 6(a), *cis/trans* 6(b), and *cis/cis* (6c). Bikker and Weaver[162] were able to show, by comparing the enthalpies of formation of the different conformations from AM1 optimization, that the *trans/trans* conformation is unfavorable. The *cis/cis* conformation, is stable and the *cis/trans* is also acceptable. It was therefore speculated[135] that at least one ester must be in the *cis* conformation for receptor binding.

Although 4-phenyl-1,4-dihydropyridine is unambiguously accepted as having a flat-boat structure, the structure of the 4-unsubstituted dihydropyridines 1-methyl-1,4-dihydronicotinamide (**59**) or NADH (**48**) is still controversial. X-ray crystallography studies[167] of the analog (**59**) indicated the 1,4-DHP ring is a planar structure. However,



(59)

it does not reflect the true conformation of (**59**) nor that of NADH (**48**) since both are in solution form. In contrast, quantum mechanical calculations[168-169] indicated a flat-boat conformation which can easily be defolded to a planar structure as the energy barrier for the unpuckering is small. Further support for the boat conformation was reported by Kellog *et al*[170] who carried out X-ray studies for a crown-ether-1,4-dihydropyridine compound.

1.7.2.0.0.0. Receptor Site for 1,4-Dihydropyridine Calcium Channel Antagonists

Little is known about the calcium channel receptor site for 1,4-DHP CCAs. Goldmann and Stoltefuss[135] proposed a lipophilic domain which interacts with the pseudoaxial phenyl moiety and the two alkyl moieties of the ester substituents (Figure 7). Hydrogen-bonding between the -NH group of the dihydropyridine ring, or the carbonyl oxygen moiety of the esters, is likely involved in binding to the active binding site. Studies[158-159] which show a correlation between calcium antagonist activity with the degree of DHP ring distortion from planarity suggest that a less flat boat-shaped 1,4-DHP ring binds more effectively to the receptor site.

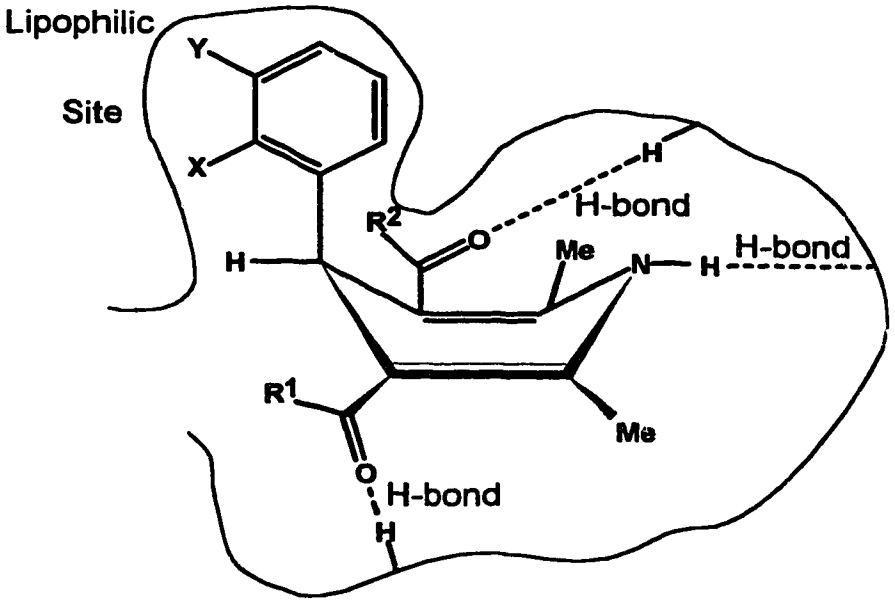


Figure 7. A putative receptor site for 1,4-dihydropyridine calcium channel antagonists.

1.7.3.0.0.0. Calcium Channel Agonist Activity of 1,4-Dihydropyridines

Ester substituents at the C-3 and C-5 positions are generally essential for 1,4-DHP CCA activity. For convenience, the left and right sides of the DHP ring system are defined as port-side and starboard-side, respectively (Figure 8). It was found that replacement of the ester group on the port-side by a nitro group such as that in BAY K

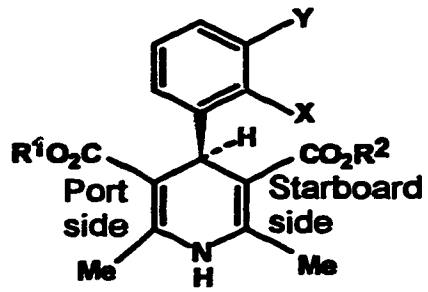
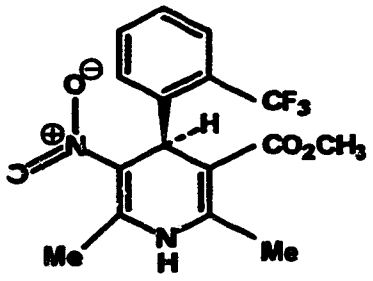
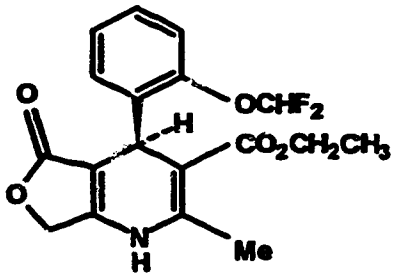


Figure 8. The assignment of port-side and starboard side to a chiral 1,4-dihydropyridine.

8644 (60), or by a lactone as in CGP 28-392 (61), produced a diametrically opposite calcium channel agonist effect due to an enhanced influx of Ca^{2+}. In contrast, replacement of the ester substituent on the starboard side by a nitro substituent, or lactone moiety, resulted in a decreased CCA activity. A study[171] using [^{3}H]-BAY K 8644, which showed that the agonist binds to a high affinity site and that the agonist drug can be displaced by nitrendipine, suggests that both calcium channel agonists and antagonists bind to the same receptor site. The mechanism, at the molecular level, by which agonist and antagonist drugs exert their effect is therefore of immense interest.



(60)



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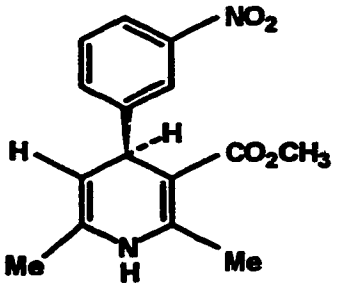


(62)

Mahmoudian and Richards[172] proposed an explanation to explain agonist/antagonist activity based on conformational analysis of the esters/nitro substituents at the C-3 and C-5 positions. Using the molecular orbital program MOPAC,

and optimization with the MNDO method, the conformational energies of both esters and nitro substituents were determined as a function of the torsional angle between the rotating substituents and the plane of the DHP-ring system. Agonists were found to possess a *trans*-directed conformation while antagonists were *cis*. He went on to propose that agonists may form a hydrogen-bond to the receptor via a *trans* directed oxygen while antagonists are *cis* directed. Differences in the domain for hydrogen-bonding may contribute to agonist/antagonist behaviour. Similar calculations shows both conformations are possible for compounds exhibiting partial agonist activity. This explanation, although attractive, does not account for the weak agonist activity exhibited by some DHPs such as (62) with a hydrogen on the port-side, nor does it account for the fact that both *cis* and *trans* hydrogen-bonding are possible for the nitro substituent of BAY K 8644.

Goldmann and Stoltefuss[135], has suggested that the effect was determined by the electron-density on the port-side of the DHP ring system. The negative charge on the oxygen of the nitro group and carbonyl oxygen of the lactone, which has a *trans* carbonyl group, is responsible for interaction with a positively charged domain on the receptor which results in channel opening. Hence, they are classified as 'Active calcium agonists'. When the negative-charged moiety is absent, the negative electrical potential exerted by the π-electron cloud of the C-4 phenyl ring can induce agonist activity towards the calcium channel although to a lesser extent. Thus, the DHP (62) which lacks an ester group on the port-side is a weak agonist and is classified as a 'Passive calcium agonist'. In contrast, compounds with a *cis* carbonyl ester on the port-side shields the phenyl π-electron cloud by the lipophilic alkyl group of the ester and at the same time binds to the lipophilic domain of the receptor and stabilizes the inactive state. They are thus classified as calcium channel antagonists.

A recent report[173] suggested a "capsized-boat hypothesis" as a putative mechanism for agonist activity. This hypothesis assumes that the DHP must possess a *cis* carbonyl on the port-side for binding to the receptor. Antagonists which fulfil this requirement would have a 'normal-boat' conformation with the phenyl ring pointing upward. The absence of a *cis* carbonyl moiety would require the ester from the star-

board side for binding. In so doing, the boat is "capsized" and the phenyl ring would be pseudoaxially pointing down. Interaction of the phenyl ring with the lower domain may be responsible for the agonist activity.

The hypotheses presented offer explanations to explain the difference between agonist and antagonist effects based on the conformation of the 1,4-DHP molecule. Little is known regarding the conformation of the receptor active site, or the nature of the drug-receptor interaction. When the amino-acid residues involved in the drug-receptor interaction with the port-side ester/nitro substituents are identified,[174] a more accurate explanation for channel gating, activation and inactivation should become possible.

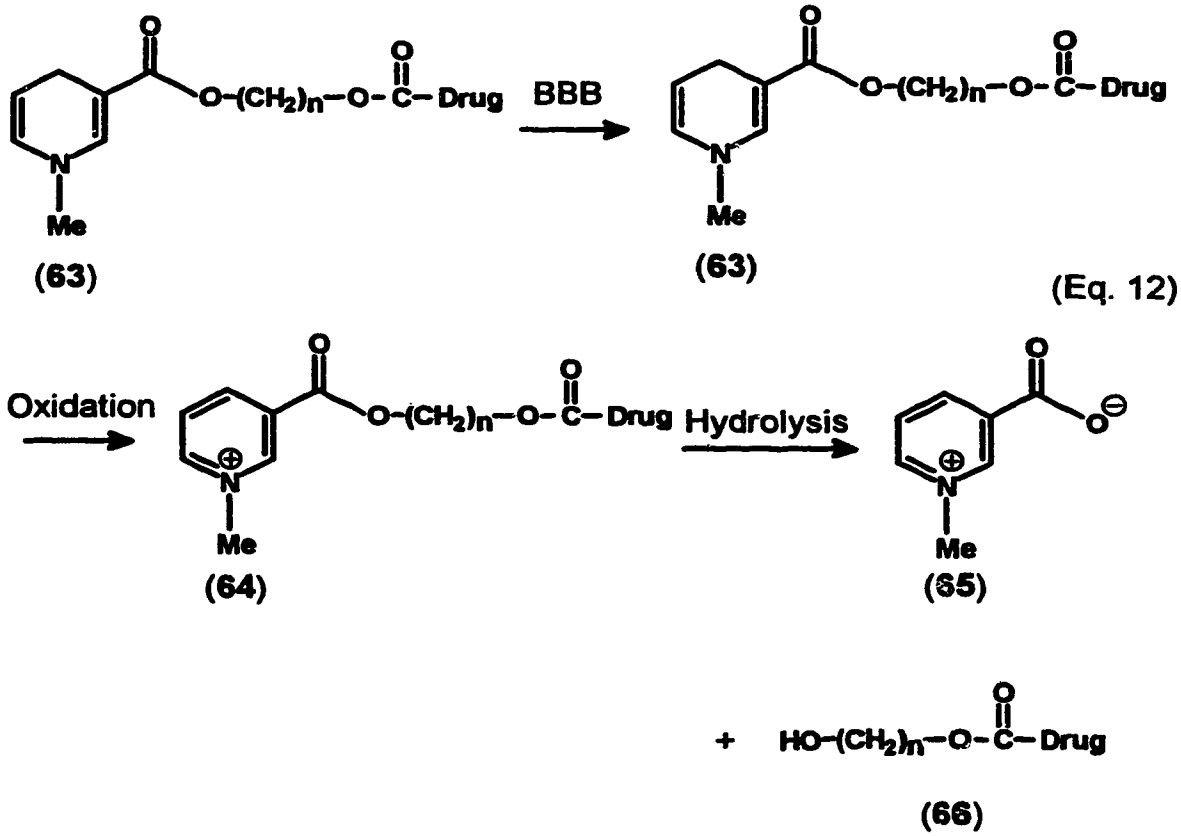
2.0.0.0.0.0. OBJECTIVES OF RESEARCH

In view of the fact that some 20 - 30%[7-9] of epileptic individuals are refractory to current medical treatment, and that many undesirable side-effects are associated with the use of conventional AEDs, the development of effective AEDs with low toxicity and a longer duration of action is a challenge to medicinal chemists.

There is now convincing evidence that Ca^{2+} is involved in the pathogenesis of seizures (see 1.4.0.0.0.). Although the role played by Ca^{2+} in this dysfunction is not fully understood, it is considered to be responsible for the intrinsic burst discharge (see 1.2.2.1.0.0.) and facilitation of the synchronization of a large number of neurons for burst firing (see 1.2.2.4.0.0.) by releasing excitatory neurotransmitters. These findings have prompted the potential use of CCAs as a novel class of AEDs. Since the late eighties, there have been numerous reports concerning the use of CCAs for inhibiting seizures induced in different experimental models. Verapamil[175] was reported to retard the rate of kindling seizures in rats, while flunarizine[176] was portrayed as a potential AED for the future. Nimodipine (**38**), which has received the most attention due to its action on the CNS, has been reported to be an efficacious anticonvulsant. For example, nimodipine was shown to protect against seizures induced by maximal electroshock (MES),[177-178] pentylenetetrazole,[96,179-180] and picrotoxin.[181] Furthermore, it was reported[179] that nimodipine raised the threshold of pentylenetetrazole induced seizures by 50-60%. Clinically,[182] nimodipine was administered in conjunction with other AEDs to 21 individuals with intractable epilepsy. It was reported that 67% of these epileptics showed a reduction of seizure frequency after a 12 week period of treatment. All of these findings show a potential for the development of CCAs as a novel class of AEDs.

The main objective of this research project was to design, synthesize and pharmacologically evaluate dihydropyridine CCAs as novel anticonvulsants. Nimodipine (**38**), and felodipine (**39**) which does not have cardiac side-effects were chosen as prototype anticonvulsants. Both compounds were synthesized as reference drugs for comparison with other DHP analogs.

Since DHPs are usually vascular-selective, the design of the DHPs is such that they are brain-targeted. It was envisaged that the undesirable hypotensive effect associated with the use of the DHPs can then be reduced. A pro-drug concept was investigated to determine whether it was a suitable method to enhance the selective brain delivery of CCA drugs. A variety of chemical delivery systems (CDS) were coupled to the alkyl chain of a C-3 alkyl ester substituent present on the parent DHP ring system. The activity of the parent DHP with and without the CDS should then be compared. The Bodor CDS, viz a 1-methyl-1,4-dihydropyridyl-3-carbonyloxy moiety as shown by (63), was studied in detail. (Eq 12). Due to its high lipophilicity, the prodrug should be selectively delivered to the brain. After entering the brain, the CDS can be oxidized to a pyridinium salt (64) similar to that of the NAD^+ ⟷ NADH redox system.



Due to the polar nature of (64), the BBB should prevent egression of the pyridinium salt. In this way, the drug is "locked in" the brain and slowly hydrolysed to liberate the active drug (66) and non-toxic trigonelline (65). In this way, a high concentration of the drug should localize in the brain and provide increased anticonvulsant efficacy. At the same time, the concentration of drug in the periphery should be low as the salt (64) should be rapidly eliminated by excretion. Thus, the peripheral cardiovascular effect should be minimal.

The successful use of 1-methyl-1,4-dihydropyridyl-3-carbonyloxy CDS prodrugs has been reported for numerous therapeutic agents such as β-lactam antibiotics,[183-184] phenytoin,[185-186] anti-HIV nucleosides,[187-189] nonsteroidal antiinflammatory drugs[190] (NSAIDs), dopamine,[191] steroids,[192] and tryptophan.[193] In these studies, improved delivery and sustained release of the parent compound in the CNS was evident.

In this research project, the DHP CCA anticonvulsant agents synthesized were submitted to the U.S. National Institute of Health (NIH), Antiepileptic Drug Development Program to determine their anticonvulsant activity in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scMet) induced seizure screens. Calcium antagonist activity was determined as the molar concentration of drug which causes a 50% decrease in the slow component (IC_{50}) in guinea pig ileal longitudinal smooth muscle (GPILSM) induced by the muscarinic agonist carbachol.

To evaluate the effectiveness of the Bodor type CDS, the felodipine and a felodipine-CDS analog were chosen for study. *In vitro* incubation of the CDS (63) with rat blood plasma or brain homogenate and then quantitative HPLC analysis was investigated to determine the rates for oxidation of the CDS (63) and hydrolysis of (64). *In vivo* studies involving the administration of the CDS (63) to rats by tail-vein injection were performed and the animals were sacrificed at selected times. The concentration (μg/g tissue) of the CDS (63), (64) and (66) were determined by quantitative HPLC analysis. The extent of brain-uptake for the CDS (63), its oxidation to the salt (64), the lifetime of the salt (64), and the rate of salt hydrolysis were determined. Similar animal studies were performed for felodipine and the data was compared to evaluate the efficacy of the felodipine CDS as a prodrug for anticonvulsant DHPs.

Since many DHPs that have been reported possess a two-carbon alkyl chain on the C-3 ester substituent, it was also of interest to determine the effect of chain length on CCA activity by introducing one-carbon and three-carbon spacers in the C-3 ester of DHPs.

Amlodipine (**36**) is a unique DHP which has a C-2 aminoethoxymethyl substituent. An analog of felodipine having a 2-hydroxyethoxymethyl substituent was synthesized. This latter compound was coupled to the 1-methyl-1,4-dihydropyridyl-3-carbonyloxy CDS and their CCA activities were compared.

Additional structure-activity relationships (SARs) were acquired by introducing various substituents at different positions on the 4-phenyl ring.

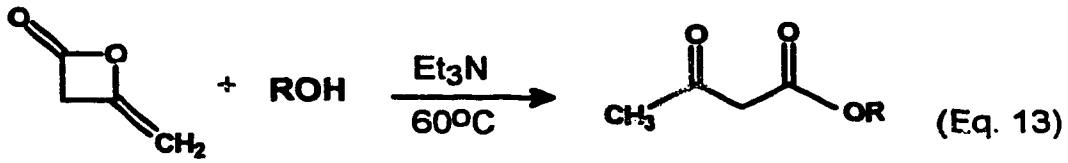
3.0.0.0.0.0. RESULTS AND DISCUSSION

3.1.0.0.0.0. SYNTHESES

3.1.1.0.0.0. Synthesis of β-ketoesters and enamine esters

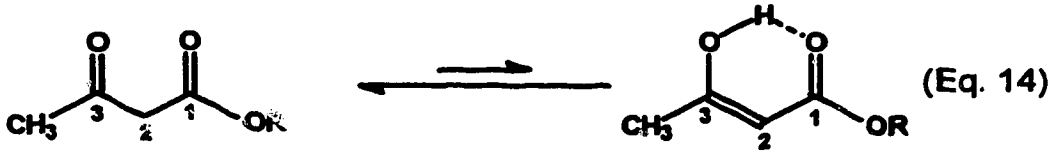
The Hantzsch reaction[136] is used extensively for the syntheses of various 1,4-DHPs. This reaction involves the condensation of β-ketoester, enamine ester and aryl aldehyde reagents (1.6.1.1.0.0.).

A β-ketoester, such as the alkyl acetoacetate (**69**) is prepared by the reaction of an alcohol (**68**) with diketene (**67**)[194] in the presence of a catalytic amount of base such as triethylamine at 60^0 C (Eq. 13).



(67) (68) (69) (Eq. 13)

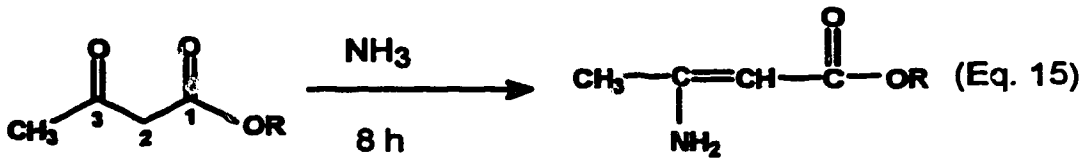
Although keto-enol tautomerism favours the formation of the keto-tautomer (**69**), the presence of the enol tautomer (**70**) can be detected by ^{1}H NMR spectrometry where the C-2 vinyl proton resonates at about $\delta = 5$. This observation is due to the fact that the enol (**70**) is stabilized by intramolecular hydrogen bonding as shown in Equation 14.



(69) (70) (Eq. 14)

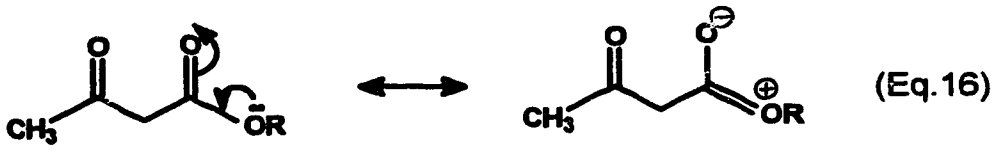
Integration of the ^{1}H NMR spectra for keto-enol tautomers (**69 - 70**) shows that the ratio of the integral for the C-2 methylene protons ($\delta \approx 3.5$) of (**69**) (integral/2), compared to that of the C-2 vinyl proton for (**70**) gives a ratio of (**70**)/(**69**) of about 1:11 (8.3% of enol tautomer **70**).

Synthesis of enamine esters such as alkyl 3-aminocrotonates (71) is readily effected by bubbling a stream of ammonia gas into a solution of the alkyl acetoacetate (69) for 8 hours at room temperature[195] (Eq. 15).

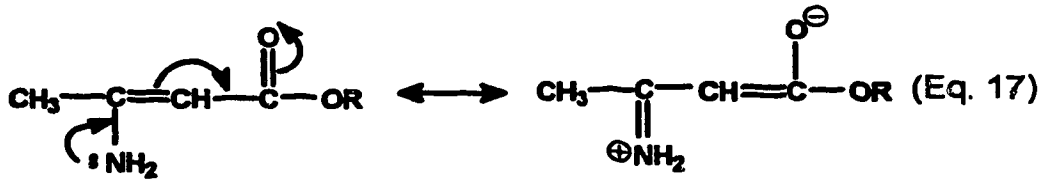


(69) (71) (Eq. 15)

The fact that ammonia reacts selectively with the β-keto group rather than the ester carbonyl in (69) is due to delocalization of the electron lone-pair from the alkoxy oxygen to the carbonyl oxygen (Eq. 16). As a result, the ester carbonyl carbon possesses a higher electron density and hence a reduction in electrophilicity which makes ammonolysis of the ester very unlikely. Furthermore, the resulting enamine moiety present in (71) is conjugated with the ester carbonyl to provide resonance stabilization as shown in Eq. 17.



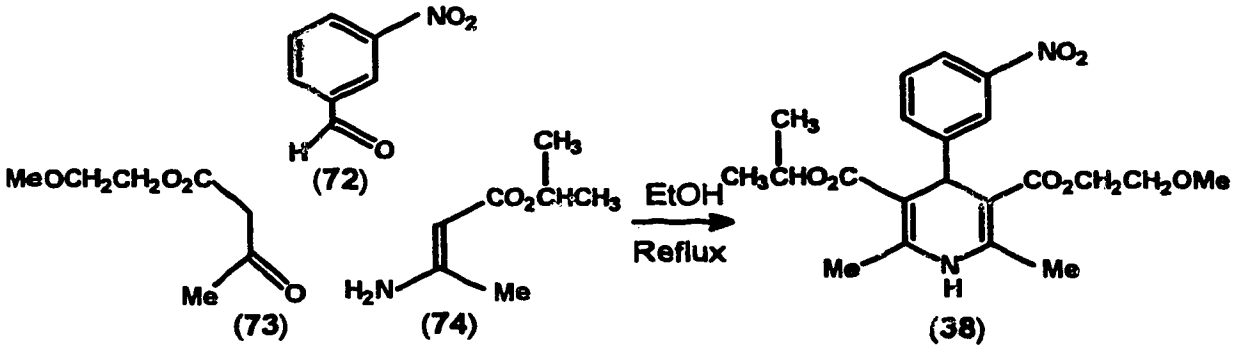
(69) (Eq.16)



(71) (Eq. 17)

3.1.2.0.0.0. Synthesis of nimodipine (38) and felodipine (39)

Nimodipine, 3-(2-methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (38) is a well-known brain-selective DHP (2.0.0.0.0.0.) whereas felodipine, 3-ethyl 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (39) is a potent vasodilator that is highly selective for vascular smooth muscle relative to myocardial tissue. Hence felodipine, unlike other CCAs, does not exhibit cardiac side-effects (1.6.0.0.0.0.). Both compounds were therefore synthesized as reference drugs for comparison with other DHP analogs investigated in this study.

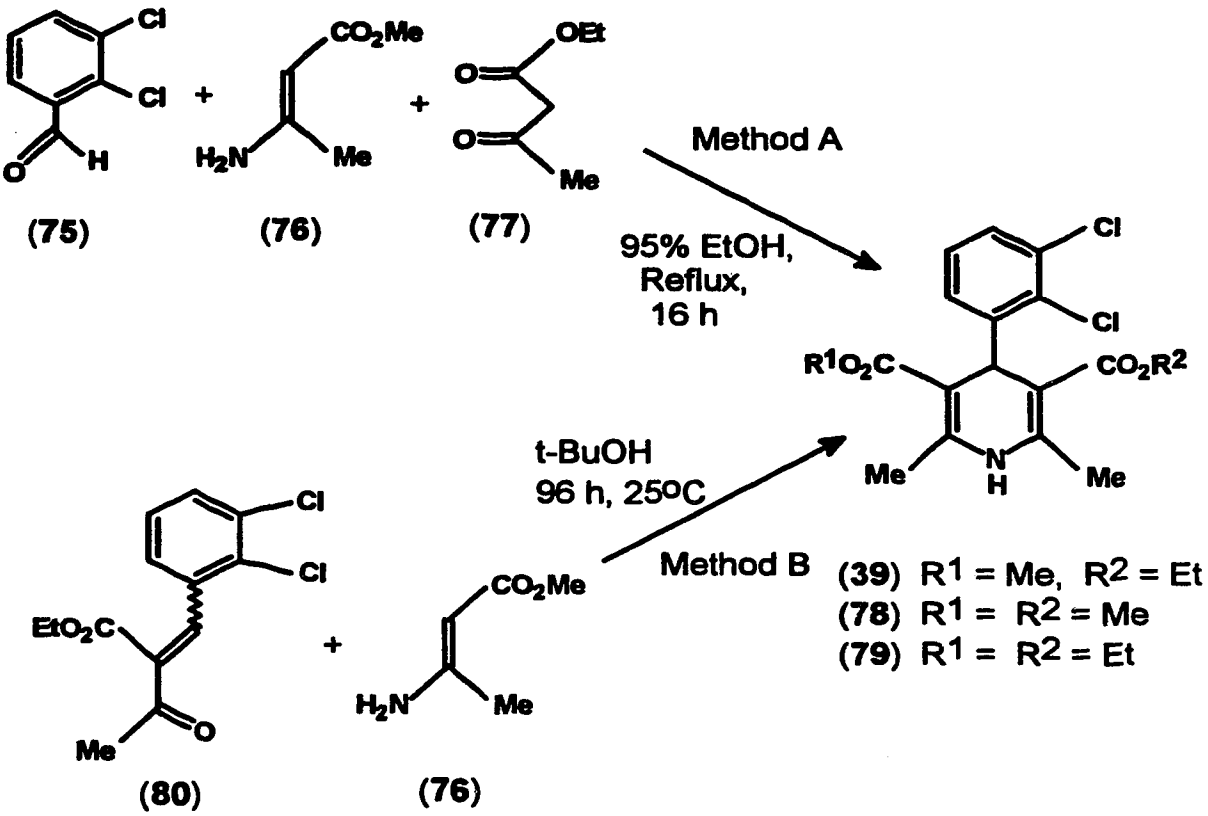


Scheme 1. Synthesis of nimodipine (38).

Nimodipine was synthesized using a 3-component reaction which involved refluxing 3-nitrobenzaldehyde (72), isopropyl 3-aminocrotonate (74) and 2-methoxy-ethyl acetoacetate (73) in 95% EtOH for 16 hours. (Scheme 1) The product nimodipine, which was purified by silica gel column chromatography and recrystallization, was obtained in 69 % yield.

A similar method was used to prepare felodipine (Scheme 2, method A) by refluxing 2,3-dichlorobenzaldehyde (75), methyl 3-aminocrotonate (76) and ethyl acetoacetate (77) in 95 % EtOH for 16 hours which yielded felodipine (39), as well as the undesired symmetrical DHPs (78) and (79).

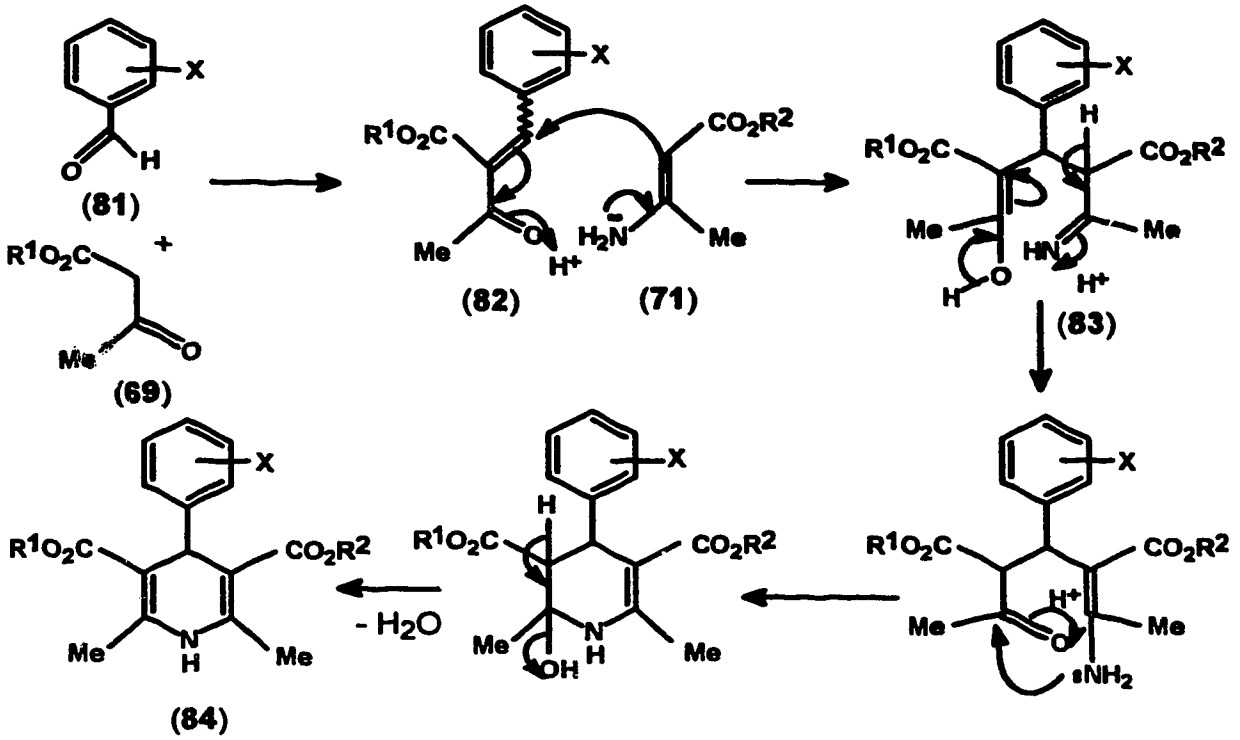
Due to their similarity in structure and polarity, neither silica gel column chromatography nor TLC successfully separated (39), (78) and (79). HPLC analysis showed that the ratio of (39):(78):(79) was 65.7:23.7:10.6.



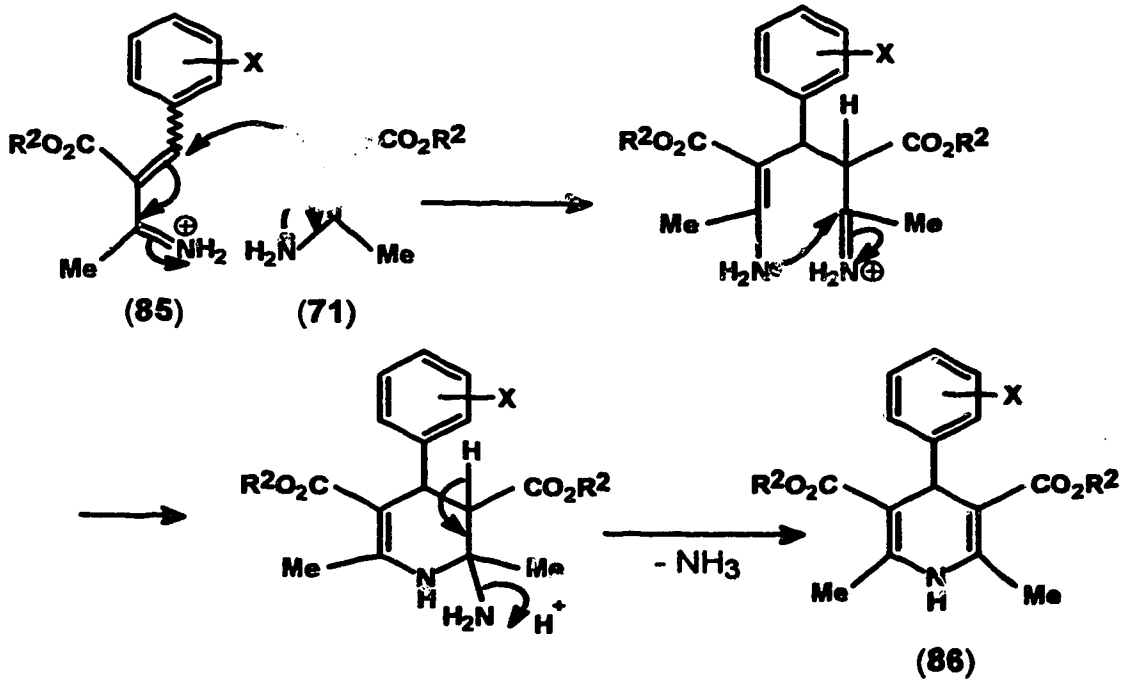
Scheme 2. Syntheses of felodipine (39).

In order to offer an explanation for the formation of these symmetrical diester side-products, the mechanism for the Hantzsch condensation proposed by Eisner and Kuthan[140] needs to be considered. The substituted-benzaldehyde (81) reacts with the alkyl acetoacetate (69) to give an alkyl 2-benzylideneacetoacetate (82) which then undergoes a Michael addition by the alkyl 3-aminocrotonate (71) to form an intermediate (83) (Scheme 3). Cyclization of this intermediate followed by loss of water forms the DHP product (84).

However, self-condensation of the intermediate (85), which is formed in the reaction, with (71) can also occur to form the symmetrical DHP (86) as shown by (Eq. 18).



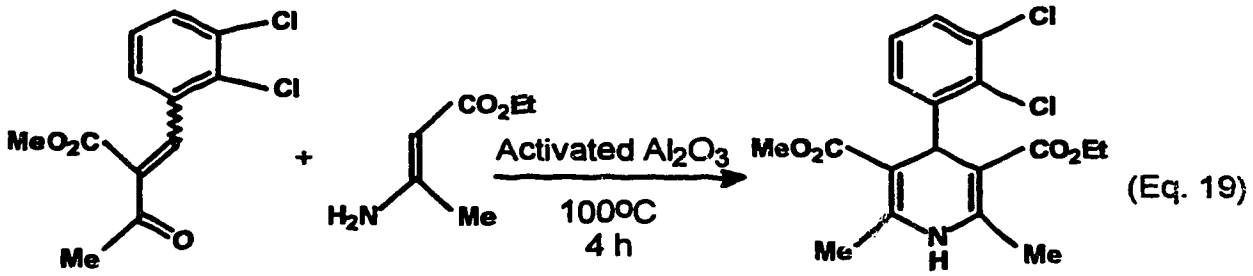
Scheme 3. Mechanism for the Hantzsch reaction.



(Eq. 18)

In order to eliminate the formation of symmetrical side-products, a modified Hantzsch reaction[196] was attempted. Ethyl 2-(2,3-dichlorobenzylidene)acetoacetate (**80**) was stirred at room temperature with methyl 3-aminocrotonate (**76**) in dry *t*-BuOH for 96 hours. (Scheme 2, method B). In this case, the product (21% yield) was shown by HPLC analysis to consist of a mixture of (**39**), (**78**) and (**79**) in a ratio of 96.6:1.6:1.8, respectively. The formation of (**78**) and (**79**) in this latter reaction may be due to a very limited hydrolysis of ethyl 2-(2,3-dichlorobenzylidene)acetoacetate (**80**) to 2,3-dichlorobenzaldehyde (**75**) and ethyl acetoacetate (**77**) by water produced in the Hantzsch condensation reaction.

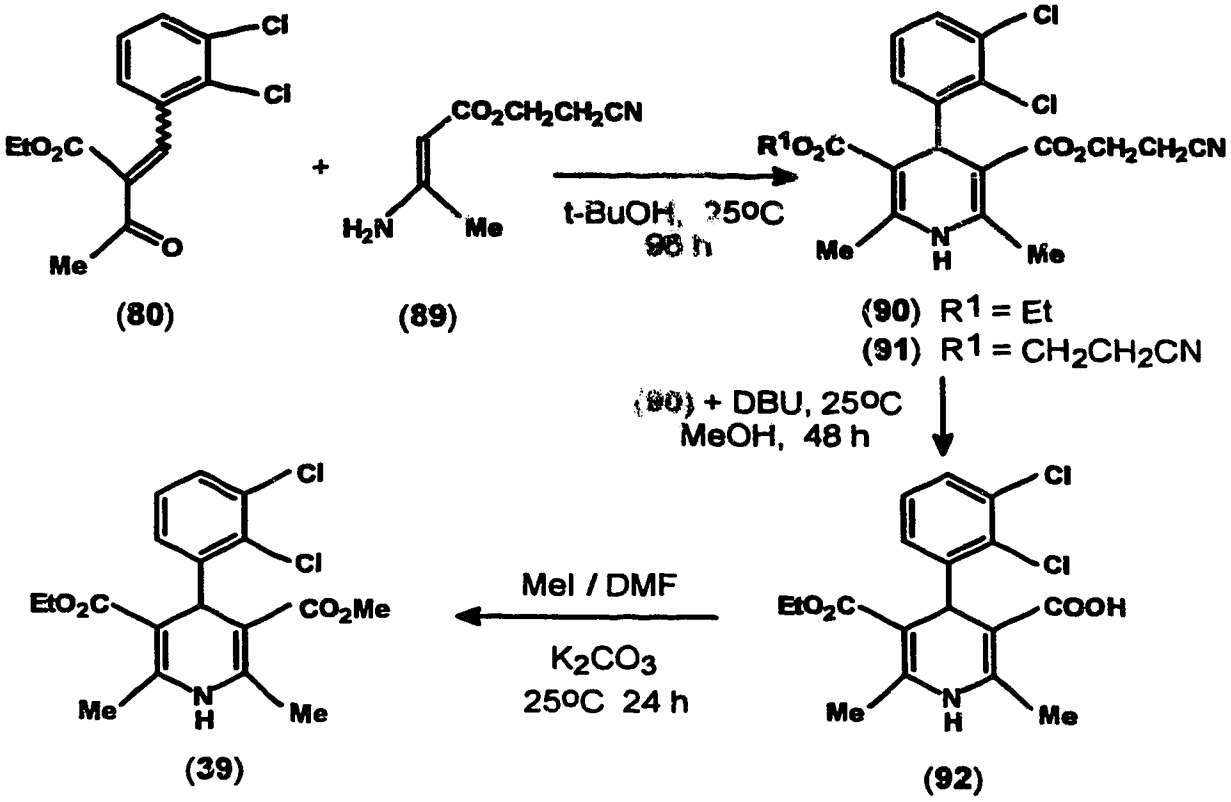
The formation of diester side-products in the synthesis of unsymmetrical DHPs is a limitation of the Hantzsch reaction.[197] An alternative no solvent synthetic method to prepare felodipine (**39**) which involved the condensation of (**87**) and (**88**) in the presence of an activated alumnina catalyst (53% yield) was recently reported[198](Eq. 19).



(87) (88) (39) (Eq. 19)

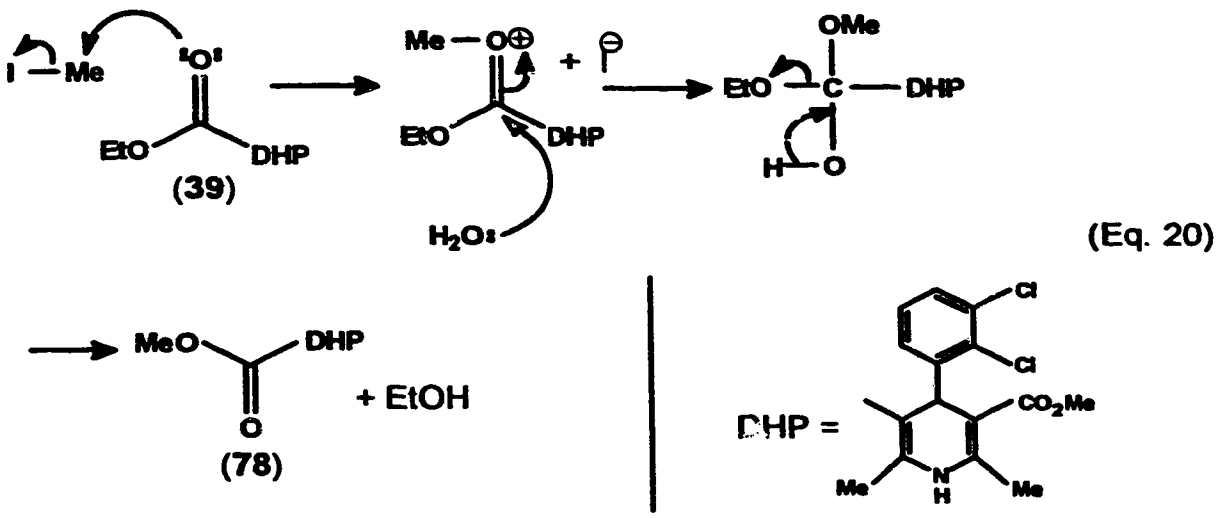
In view of our inability to obtain pure felodipine using the procedures described, an improved synthetic method was developed (Scheme 4). It was envisaged that the use of a more polar ester substituent such as a 3-(2-cyanoethyl) ester, that could be readily converted to the carboxy analog using a non-nucleophilic base promoted β-elimination of acrylonitrile, would circumvent this problem. Thus, condensation of ethyl 2-(2,3-dichlorobenzylidene)acetoacetate (**80**) with 2-cyanoethyl 3-aminocrotonate (**89**) afforded a mixture of the unsymmetrical 3-(2-cyanoethyl) 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**90**, 71%) and the symmetrical 3,5-*bis*-(2-cyanoethyl) product (**91**, 6.1%) that were readily separated by silica gel column

chromatography (Scheme 4). Cleavage of the unsymmetrical ester (**90**) using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave 3-ethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**92**, 73%) which was then converted to high-



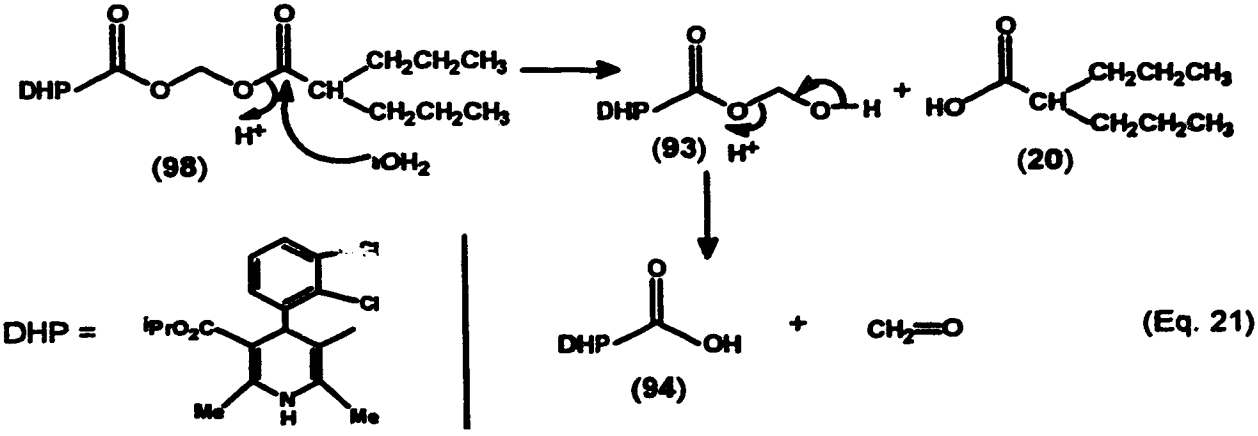
Scheme 4. An improved synthetic method to prepare high purity felodipine (**39**).

purity felodipine (**39**) by reaction with iodomethane in 70% yield. HPLC analysis indicated that felodipine prepared using this procedure contained a trace quantity of the symmetrical dimethyl ester (**78**) where the ratio of (**39**):(**78**) was 99.3:0.7. The formation of (**78**) in this latter methylation reaction may be due to transesterification of the ethyl ester moiety to a methyl ester substituent, in spite of the fact that dry DMF was used as solvent. A postulated mechanism for this transesterification process is shown in Equation 20.

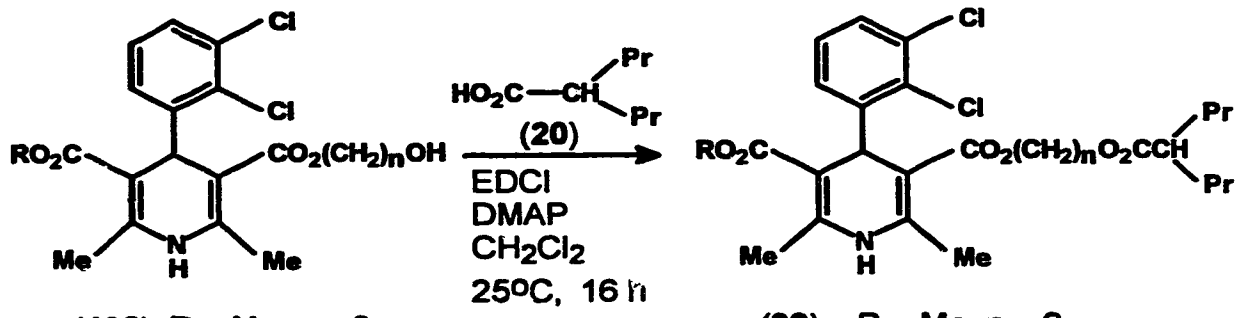


3.1.3.0.0.0. Synthesis of 3-(2-*n*-propylpentanoyloxyalkyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (98 - 101)

Since valproic acid (2-propylpentanoic acid) (20) is a potent anticonvulsant agent used for the treatment of absence, myoclonic and tonic-clonic seizures (1.3.1.9.0.0.), a prodrug in which valproate (2-propylpentanoyloxy moiety) was coupled to a DHP moiety was designed. It was envisaged that hydrolysis of an ester such as (98) would liberate valproate and hence provide protection against various seizures. More particularly, the lipophilic DHP moiety may enhance CNS penetration to provide elevated and prolonged brain levels of valproate.



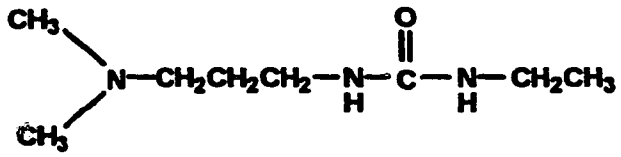
It was also of interest to vary the chain length of the C-3 ester substituent in this series of compound. A 3-carbon chain compound, n = 3 (**101**) was introduced and this effect on anticonvulsant activity was determined. The single carbon spacer DHP (**98**) was synthesized as a double ester prodrug.[199] It was envisaged that after uptake in the brain, (**98**) would be hydrolysed to liberate valproic acid (**20**) and an unstable hydroxymethyl ester (**93**) which would break down rapidly releasing formaldehyde and the DHP drug (**94**) (Eq. 21).



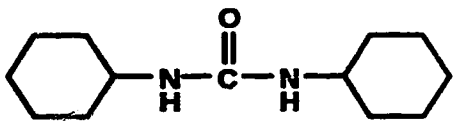
(**102**) R = Me n = 2
(**103**) R = iPr n = 2
(**104**) R = iPr n = 3

(**99**) R = Me n = 2
(**100**) R = iPr n = 2
(**101**) R = iPr n = 3

Scheme 5. Syntheses of 3-(2-*n*-propylpentanoyoxyalkyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (**99-101**).



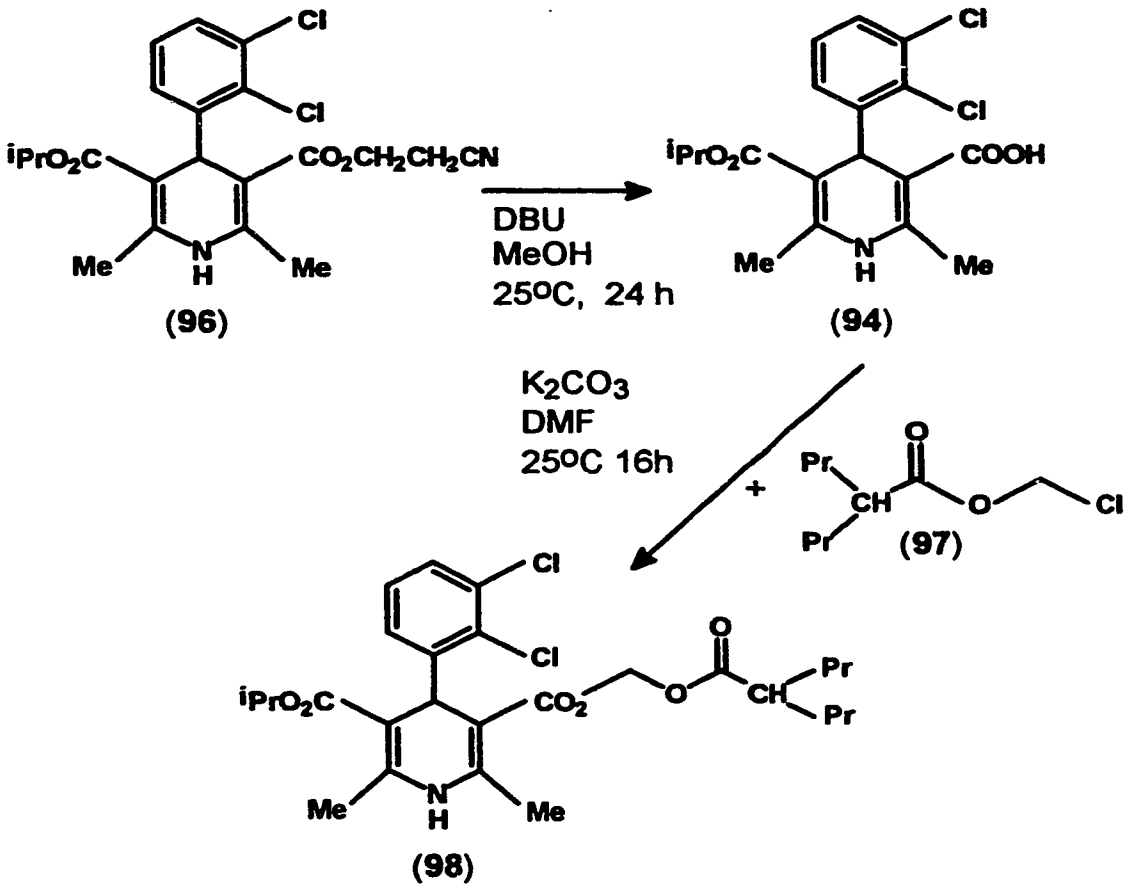
(**105**)



(**106**)

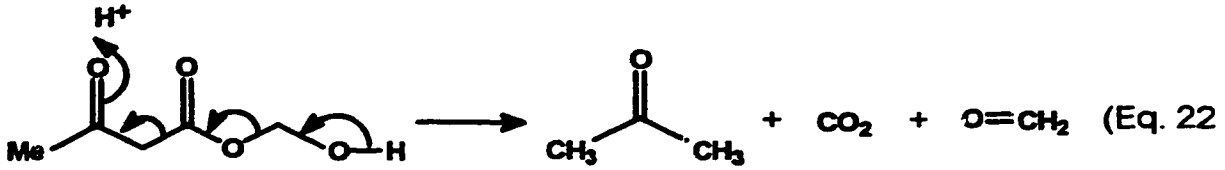
Compounds of this type (**99 - 101**) were prepared by esterification of the corresponding hydroxyalkyl 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (**102 - 104**) with 2-propylpentanoic acid (**20**) (Scheme 5) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 for 16 hours. Purification of the product afforded (**99 - 101**) in 63 - 95 % yields. The reason for choosing EDCI[200], rather than 1,3-dicyclohexylcarbodiimide (DCC) in this esterification reaction is because

the former reagent gives 1-(3-dimethylaminopropyl)-3-ethylurea (**105**) after reaction. Since (**105**) is basic, it can be easily removed by washing with acid. Although DCC is converted to 1,3-dicyclohexylurea (**106**) which precipitates, it is slightly soluble in organic solvents making it difficult to remove.



Scheme 6. Synthesis of 3-(2-*n*-propylpentanoyloxymethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**98**).

Compounds such as (**98**), that possess a one carbon spacer, required an alternate synthetic procedure for their synthesis since hydroxymethyl acetoacetate (**95**) was expected to eliminate formaldehyde and carbon dioxide rapidly during the Hantzsch synthesis (Eq. 22).



(Eq. 22

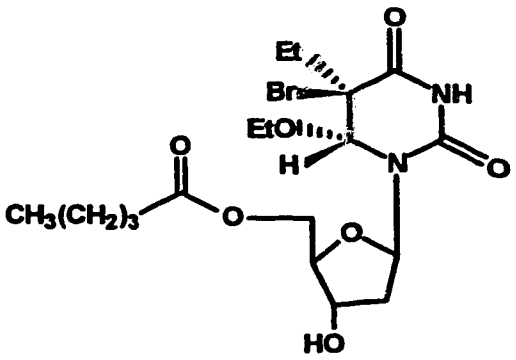
(95)

An alternative synthesis was therefore devised to prepare compounds such as (98). Thus cleavage of 3-(2-cyanoethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (96) using DBU yielded isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (94) (80%). Alkylation of (94) with (97) afforded (98) in 97% yield (Scheme 6).

3.1.4.0.0.0. Synthesis of 3-(2-pentanoyloxyethyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (108 - 109)

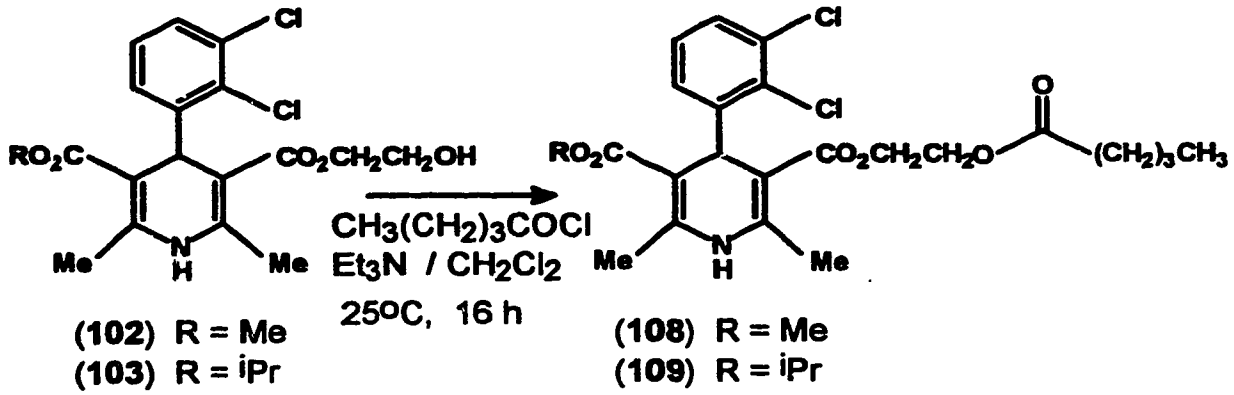
Since epileptic seizures originate in the brain (1.2.2.0.0.0. - 1.2.2.4.0.0.), an effective anticonvulsant drug must be brain-targeted to provide an effective brain concentration. The entry of drugs into the brain is often limited by their inability to cross the blood brain barrier (BBB)[201] which by its nature excludes the passage of many polar compounds, although essential amino acids and glucose can gain access via active-transport mechanisms. In designing a brain-targeted drug, lipophilicity must be taken into consideration. The lipophilicity of a compound is reflected by its partition coefficient (Kp) which is a physicochemical parameter defined as the concentration ratio for a compound that partitions between *n*-octanol and water. A larger Kp value enhances a compound's ability to dissolve in membrane lipids. It is well documented that a compound requires a Kp value of about 100 for efficient passage across the BBB. For example, Bundgaard and Buur,[202] in a study to increase the bioavailability of 5-fluorouracil, assessed a series of 1-alkoxycarbonyl derivatives as possible CDS to protect the drug from first pass metabolism in the GI tract and liver. It was anticipated that the prodrug approach would increase lipophilicity and that this improved permeability across

membranes may protect the parent drug from first-pass metabolism. In this study, it was found that the 1-butoxycarbonyl moiety improved the bioavailability of 5-fluorouracil by 100% with a 50-fold increase in lipophilicity suggesting a four-carbon alkyl chain provides the optimal chemical delivery system for 5-fluorouracil. A study by Ghosh and Mitra[203] revealed that the 5'-O-valerate prodrug ester of 5-iodo-2'-deoxyuridine possessed an increased lipophilicity and enhanced protein-binding in rat blood plasma relative to 5'iodo-2'-deoxyuridine. Based on this finding, Wiebe *et al* [204] utilized the valerate ester double prodrug of (+)-*trans*-(5R,6R)-5-bromo-5-ethyl-6-ethoxy-5,6-dihydro-5'-O-valeryl-2'-deoxyuridine (**107**) to enhance stability and potency of the antiviral agent 5-ethyl-2'-deoxyuridine. In this thesis research project, the valerate ester prodrugs (**108 - 109**) were synthesized and evaluated as anticonvulsant agents.



(**107**)

The valerate esters (**108**) and (**109**) were prepared by acylating the 3-(2-hydroxyethyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridine-dicarboxylates (**102 - 103**) with valeryl chloride in the presence of triethylamine in yields of 98 and 95%, respectively (Scheme 7).



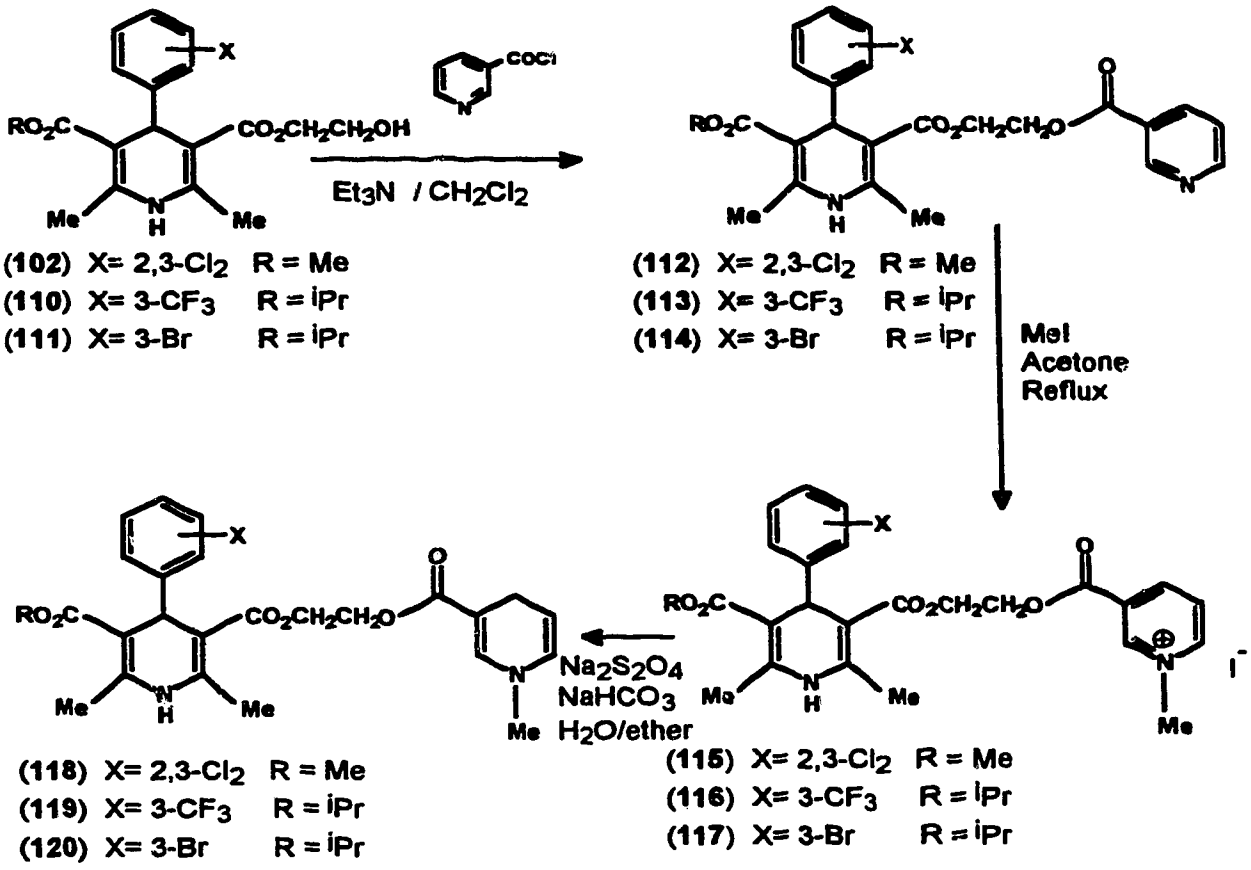
Scheme 7. Syntheses of 3-(2-pentanoyloxyethyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (**108 -109**).

3.1.5.0.0.0. Synthesis of 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (118 - 120) and 3-ethyl 5-methyl 1,4-dihydro-2-[2-[(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethoxy]methyl]-6-methyl-4-(2,3-dichlorophenyl)-3,5-pyridine-dicarboxylate (131)

Bodor[205], in an effort to improve the delivery of drugs into the brain, designed a 1,4-dihydropyridine redox chemical delivery system (CDS) similar to the $NAD^+ \rightleftharpoons$ NADH coenzyme system. The merit of this CDS is that it increases the lipophilicity of polar drugs to improve their ability to cross the BBB and provide higher brain concentrations. Oxidation of the drug-CDS to the pyridinium salt prevents egression prior to hydrolysis to release the drug in brain. This Bodor concept therefore has the potential to provide higher brain concentrations of the active drug for a longer duration.

This CDS system was therefore investigated as a method to provide a higher DHP drug concentration in brain for a longer period of time. The felodipine-CDS compound (**118**) was synthesized for *in vitro* incubation studies with rat plasma and brain homogenate, and for *in vivo* studies in rats. Amlodipine (**36**) is a potent antihypertensive drug with a long duration of action (1.6.0.0.0.0.). A structurally related

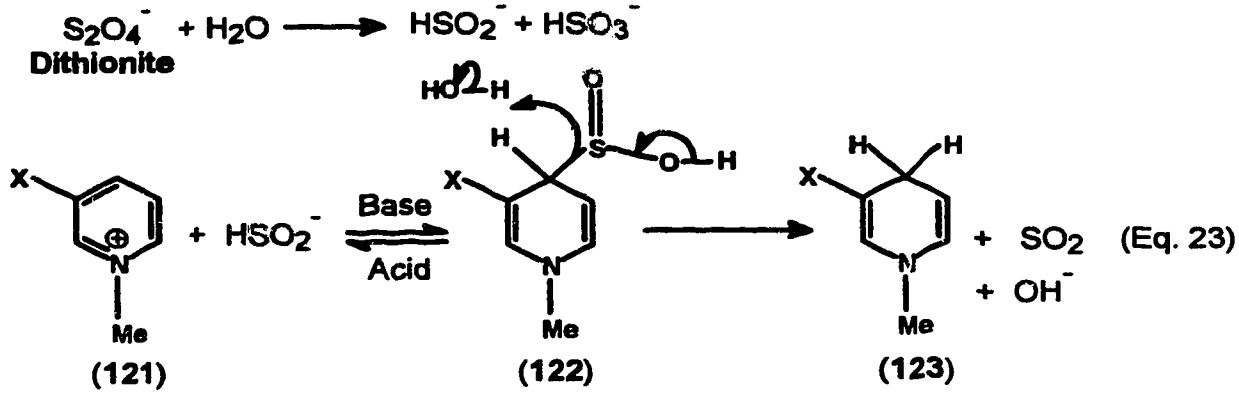
compound with the CDS-moiety attached to the C-2 substituent (**131**) was therefore synthesized for pharmacological evaluation.



Scheme 8. Syntheses of the CDS-prodrugs (**118-120**).

The syntheses of the targeted compounds (**118 - 120**) are outlined in Scheme 8. The 3-(2-hydroxyethyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (**102, 110 - 111**) were acylated with nicotinoyl chloride in the presence of triethylamine at 25°C. The nicotinoyloxyethyl esters (**112 - 114**) prepared in this way were quaternized by reaction with iodomethane in dry acetone to yield the corresponding pyridinium salts (**115 - 117**). Regioselective reduction of the pyridinium salts using sodium dithionite under alkaline conditions using a two-phase solvent system (H_2O - ether, 1:1, v/v) afforded the corresponding 1-methyl-1,4-dihydropyridyl-3-carbonyloxy derivatives (**118 - 120**) in 83 - 97% yields. The merit of using ether as the

organic phase is to extract the product as soon as it is formed but at the same time keeps the pyridinium salts (**115 - 117, 130**) in the aqueous medium since ionic compounds are not soluble in it. The mechanism for this type of sodium dithionite reduction, which has been studied[206], is shown in Equation 23.

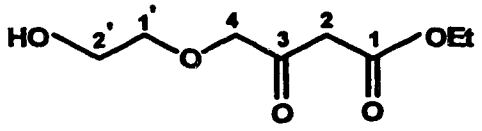


The dithionite anion reacts selectively with the pyridinium ion (**121**) by attack at the C-4 position forming the intermediate (**122**). Since this reaction is reversible, maintaining an alkaline pH is essential to ensure that the reaction proceeds in the forward direction and concurrently to prevent hydration across the C-5 - C-6 double bond which occurs readily at acidic pH[207] (1.6.2.2.0.0.). The 1,4-dihydropyridine product (**123**) is produced upon elimination of sulphur dioxide from (**122**) (Eq. 23).

Since the 1-methyl-1,4-dihydropyridyl-3-carbonyloxy CDS moiety does not possess a phenyl substituent at its C-4 position, nor an electron-withdrawing substituent at the C-5 position, this type of compound is very vulnerable to oxidation by air to give the pyridinium salt.[144] For this reason, the DHP-CDS compounds prepared (**119, 120, 131**) were not recrystallized, except for (**118**) which crystallized rapidly from methanol. The sodium dithionite reduction was carried out by stirring the DHP-pyridinium salt in the presence of a mild base such as $NaHCO_3$ using a two-phase solvent system (ether and degassed water) under an argon atmosphere. The 1-methyl-1,4-dihydropyridyl-3-carbonyloxy CDS compound is extracted immediately into the ether phase after its formation. Separating the ether layer followed by washing with degassed water, drying and evaporating the solvent gave a high yield of the products. Further purification by

silica gel column chromatography, or recrystallization, were not carried out since the CDS moiety decomposes in solution and especially during silica gel column chromatography. Compound (**118**), which recrystallized rapidly from methanol, was obtained as a crystalline product.

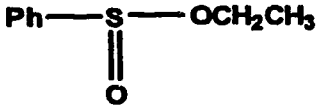
Compound (**131**) which has the CDS moiety attached to the C-2 position of the parent 1,4-dihydropyridyl ring system was prepared by the synthetic method shown in Scheme 9. Reaction of ethylene glycol with 3,4-dihydro-*2H*-pyran in THF in the presence of *p*-toluenesulphonic acid afforded the mono-protected 2-tetrahydropyranyl ether derivative (**124**, 68%) together with the *bis*-tetrahydropyranyl derivative and unreacted glycol which were separated by column chromatography. Deprotonation of (**124**) using sodium hydride and then reaction with ethyl 4-chloroacetoacetate (**125**) in THF gave the intermediate product (**126**) which without isolation, was hydrolysed using acid to afford ethyl 4-(2-hydroxyethoxy) acetoacetate (**127**, 63.8%). The condensation of (**127**) with methyl 2-(2,3-dichlorobenzylidene)acetoacetate (**87**) and ammonium acetate yielded (**128**, 32%). The subsequent reaction of (**128**) with nicotinoyl chloride in the presence of Et_3N gave (**129**, 85%) which on methylation with iodomethane afforded the pyridinium salt (**130**, 95%). Reduction of (**130**) using sodium dithionite yielded the target product (**131**, 95%) as illustrated in Scheme 9.



(**127**)

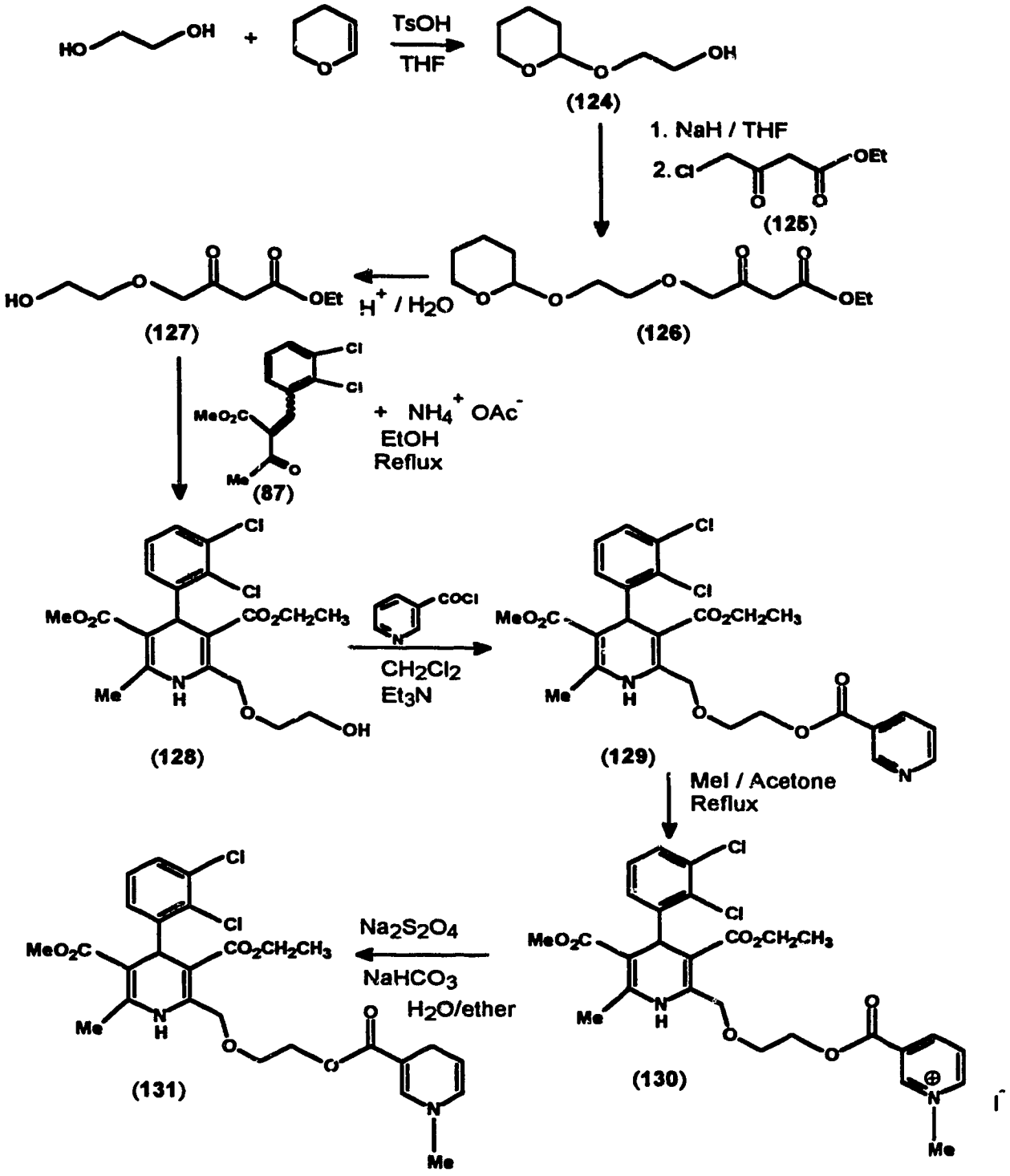
The ^1H NMR spectrum for the intermediate compound (**127**) showed non-equivalent methylene protons. For example, the C-2 methylene protons appeared as two doublets at δ 2.47 and 2.63 ($\Delta\delta$ = 0.16) with a geminal coupling constant of 15.35 Hz that is typical for a sp^3 carbon (J_{gem} = 12 - 15 Hz). The methylene protons at the C-4 position were also non-equivalent. The latter protons resonated at δ 3.71 and 3.43 ($\Delta\delta$ = 0.28) with a coupling constant of 11.25 Hz. In addition, the C-1' methylene protons also appear to be non-equivalent. This A_2BC type of spin system for the C-1' and C-2' protons makes interpretation difficult.

The reason for the non-equivalence of these methylene protons for (**127**) is interesting since there is no asymmetric carbon present. One potential explanation is the formation of an eight-membered intramolecular H-bond ring system involving the hydroxyl proton and the C-3 carbonyl oxygen atom. The observation that the hydroxyl proton appeared as a broad singlet at δ 4.92 supports this explanation and a slow rate of hydroxyl exchange. A report[231] indicated that the presence of an asymmetric centre may not be a prerequisite for non-equivalence of methylene protons since the ethoxy methylene protons of (**168**) had a chemical shift difference of 0.434 and coupled to the CH_3 protons to form an ABX_3 spin system.

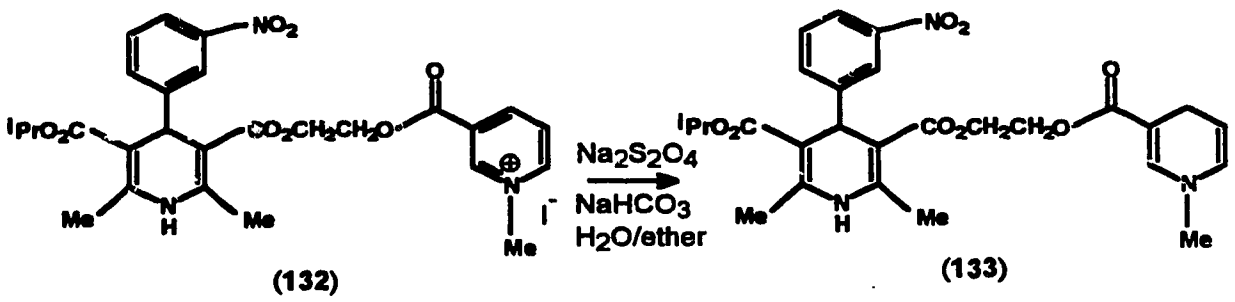


(**168**)

It was anticipated that the nimodipine-CDS compound (**133**) could be prepared using a synthetic sequence similar to that used to prepare (**118 - 120**) as outlined in Scheme 10. However, it was found that the 3-nitrophenyl substituent, which may act as an oxidizing agent, complicated the sodium dithionite reduction. As a result, a very low yield (~ 1%) of the desired product (**133**) was isolated which decomposes on storage. This instability may be due to a redox reaction between the nitro group on the phenyl ring and the 1-methyl-1,4-dihydropyridyl-CDS moiety. In contrast compounds (**118 - 120**) possessing 2,3-dichlorophenyl, 3-trifluoromethylphenyl and 3-bromophenyl C-4 substituents described earlier (see Scheme 8) were stable products.

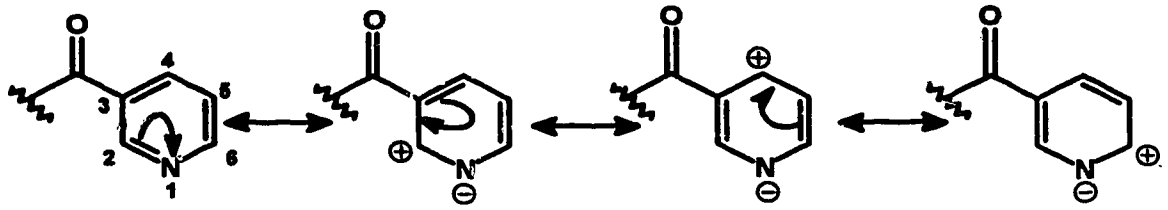


Scheme 9. Synthesis of CDS prodrug for (131).



Scheme 10. Unsuccessful attempted synthesis of the nimodipine-CDS (133).

^{1}H NMR spectroscopy showed that the resonances for the nicotinoyl protons present in compounds (112 - 114) and (129) appeared in the following order going from low to high field: H-2 > H-6 > H-4 > H-5. The low field position of the H-2, H-6 and H-4 nicotinoyl chemical shifts is due, at least in part, to resonance effects as illustrated below (Eq. 24).

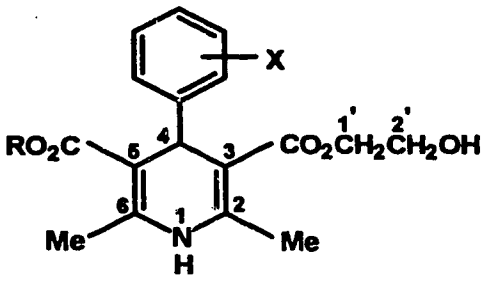


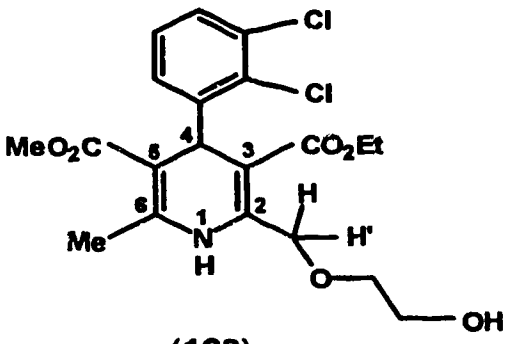
(Eq. 24)

The low electron density at the C-2, C-6 and C-4 positions results in a strong deshielding of protons attached to these positions. The fact that H-2 and H-6 are more deshielded than H-4 is due to additional deshielding induced by the negative inductive effect of the pyridine nitrogen. In additon, H-2 is more deshielded than H-6 due to the proximity of the electron-withdrawing C-3 carbonyl moiety.

Quaternization of the pyridyl nitrogen deshields the ring protons an additional 0.5 - 1 δ due to the positively charged ring nitrogen atom where the order of the chemical shifts going from low to higher field is: H-6 > H-2 > H-4 > H-5. The fact that H-6 is more deshielded than H-2 is not clearly understood.

Examination of the ^{1}H NMR spectra of (**118 - 120, 131**) showed that the chemical shifts for the 1,4-dihydropyridyl moiety are H-2 (δ = 6.9) > H-6 (δ = 5.6) > H-5 (δ = 4.75) > H-4 (δ = 3.05) which is consistent with chemical shifts for the 1,4-dihydropyridine ring system.[140] The fact that H-5 is more deshielded than H-4 is because the former is a vinyl proton which is deshielded by the π-electron of the C-5 to C-6 carbon-carbon double bond while the latter is not deshielded due to its attachment to a sp^3 carbon. Further examination showed that the 4-methylene protons appears as a broad singlet. This broadening of the H-4 resonance may be due to a conformational equilibrium between planar and boat conformations for the 1-methyl-1,4-dihydropyridyl ring[208] which provides further support for a rapid equilibrium between two conformations for the NADH 1,4-dihydropyridine model.[168-169]





(102) X = 2,3-Cl_2, R = Me
(110) X = 3-CF_3, R = ^{i}Pr
(111) X = 3-Br, R = ^{i}Pr

(128)

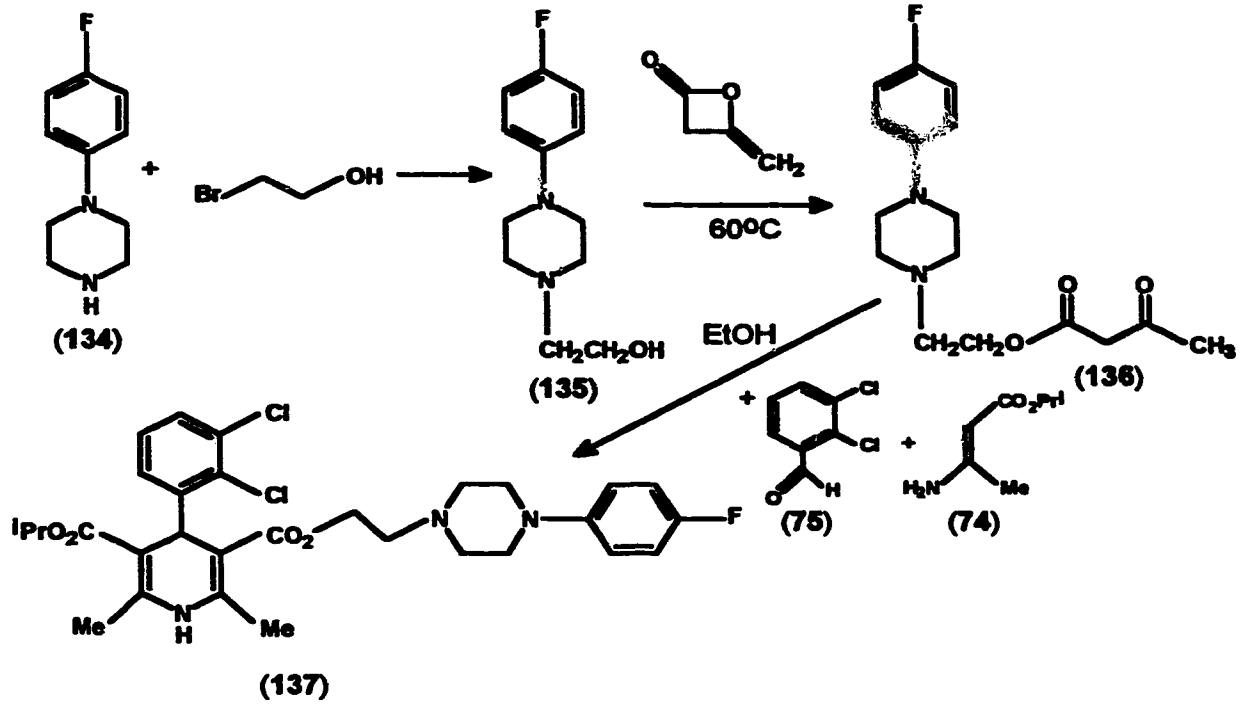
Compounds (**102**), (**110**), (**111**) and (**128**) possess an asymmetric carbon at the C-4 position. The ^{1}H NMR spectrum for compounds (**110**) and (**111**) showed that the non-equivalent C-1' protons appear as two octets at δ 4.07 and 4.26, even though the C-1' hydrogen is 5 bonds from the asymmetric centre. These two octets are the result of geminal coupling between the two non-equivalent C-1' methylene protons and their subsequent vincinal coupling to the two adjacent C-2' protons. A similar non-equivalence for the geminal methylene protons of the C-2 methoxy H and H' protons of (**128**) was also observed. Due to geminal coupling, these latter protons form an AB quartet at δ 4.82 and 4.73 with a geminal coupling constant of 16.7 Hz.

In contrast, the ^{1}H NMR spectrum for (102) did not show non-equivalent C-1' protons, although an asymmetric centre is also present at C-4. The C-1' and C-2' protons of (102) form an AA' XX' spin system that appears as two distorted triplets or two closely spaced overlapping doublets at δ 4.17 and 3.77, respectively. There was no geminal coupling between the C-1' methylene protons. This difference could be due to the presence of the $CHMe_2$ moiety that is present in (110) and (111), but that is absent in (102).

3.1.6.0.0.0. Synthesis of 3-[2-[4-(4-fluorophenyl)piperazinyl]ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (137)

Flunarizine, which has two 4-fluorophenyl substituents and a piperazine ring in its structure, is a cinnarizine type of CCA (1.5.0.0.0.0.). It is a central-acting drug that is marketed in Canada as an anti-migraine agent (1.5.1.0.0.0.). It was therefore envisaged that the presence of these moieties may enhance the delivery of DHP CCAs to the brain. Compound (137) which possesses a 1-(4-fluorophenyl)piperazinyl moiety, was synthesized and tested as an anticonvulsant agent.

The synthetic procedure employed to prepare the target compound (137) involved the initial reaction of 1-(4-fluorophenyl)piperazine (134) with 2-bromoethanol to yield 2-[4-(*p*-fluorophenyl)piperazin-1-yl]ethanol (135, 55%). Condensation of (135) with diketene at 60° C afforded 2-[4-(*p*-fluorophenyl)piperazin-1-yl]ethyl acetoacetate (136, 85%). The modified Hantzsch condensation of (136) with 2,3-dichlorobenzaldehyde (75) and isopropyl 3-aminocrotonate (74) in ethanol yielded (137, 43%) (Scheme 11).

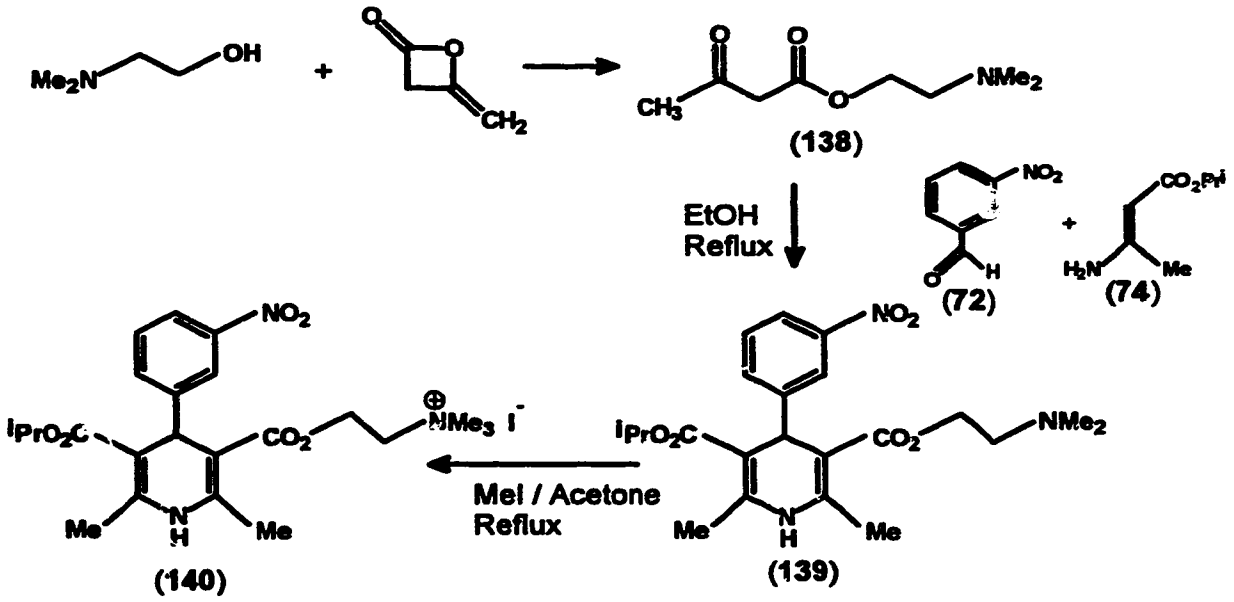


Scheme 11. Synthesis of 3-[2-[4-(4-fluorophenyl)piperazinyl]ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridine-dicarboxylate (137).

3.1.7.0.0.0. Syntheses of 3-(2-dimethylaminoethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (139) and 3-[2-(trimethyl-ammonium)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate iodide (140)

In order to probe the DHP binding site of L-type calcium channels, Triggle *et al* [209] designed a series of compounds having a quaternized ammonium group attached to the ester of the DHP moiety using a polymethylene chain spacer. It was postulated that the quaternized ammonium group, by virtue of its positive charge, would not be able to pass through the lipid-bilayer. The quaternary ammonium group was expected to act as an anchor at the plasma membrane allowing the DHP moiety attached via the hydrocarbon spacer to gain access to its DHP receptor binding site. These binding studies, which were performed using rat heart cells, indicated that an eight-carbon spacer

was the minimum chain length for maximal binding which suggests that the DHP binding site was located on the extracellular side of the lipid-bilayer. These results prompted us to synthesize (139) that has a 3-(2-dimethylaminoethyl) substituent that may facilitate its delivery and entry into brain. Although a two-carbon spacer between the ester oxygen and the NMe_2 moiety was used, it was hoped that the L-type calcium channel receptor in brain may not be the same as that in heart. It was also expected that an eight-carbon spacer would make the compound too lipophilic for *in vivo* use. It was envisaged that the 2-dimethylaminoethyl substituent of DHP (139) would be protonated at physiological pH and that it could interact with a negatively-charged domain on the receptor similar to that of amlodipine.[210] The trimethylammoniumethyl DHP analog (140), which possesses a postively charged ammonium group would help to reinforce the above hypothesis of ionic interactions by comparing its anticonvulsant activity to that of the 3-[2-dimethylaminoethyl] compound. (139).

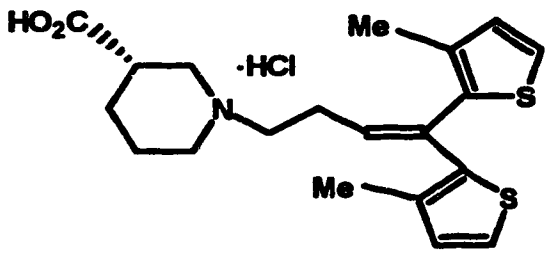


Scheme 12. Syntheses of 3-(2-dimethylaminoethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (139) and 3-[2-(trimethylammonium)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate iodide (140).

The reaction of N,N-dimethylethanolamine with diketene afforded 2-(N,N-dimethylamino)ethyl acetoacetate (**138**, 84.3%) which on subsequent reaction with 3-nitrobenzaldehyde (**72**) and isopropyl 3-aminocrotonate (**74**) in ethanol gave (**139**) in 61 % yield. Reaction of the 2-dimethylaminoethyl compound (**139**) with iodomethane in dry acetone gave the 2-trimethylammoniumethyl iodide product (**140**) in 99% yield (Scheme 12).

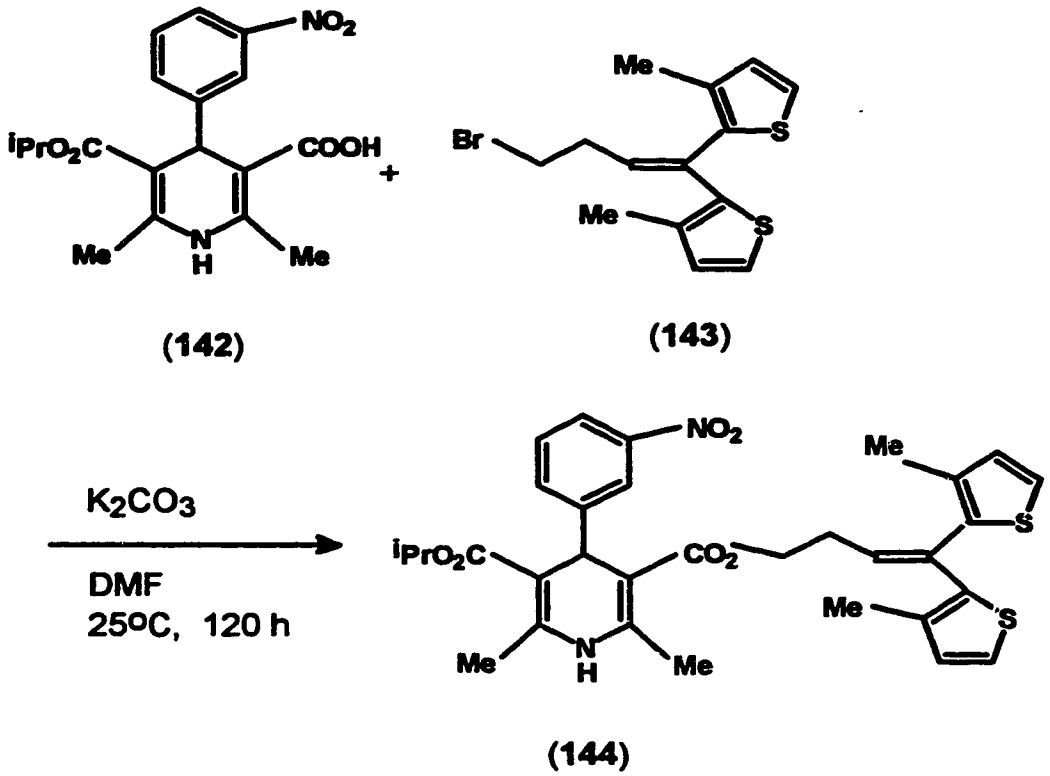
3.1.8.0.0.0. Synthesis of 3-[4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (144) and 3-[4,4-*bis*-(*o*-tolyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl) -3,5-pyridinedicarboxylate (148)

Knutsen *et al*[211] designed a series of N-substituted 4,4-diaryl/heteroaryl-3-butenyl derivatives of nipecotic acid and guvacine to acquire structure-activity relationships for this novel class of compounds which were expected to cross the BBB and act as GABA reuptake inhibitors to provide anticonvulsant activity. The polar character of nipecotic acid and guvacine, which are amino acids, restricts their entry into brain upon *in vivo* administration. In this study, it was observed that the most potent GABA reuptake inhibitor compounds were those having an *ortho*-substituent on one or both of the aryl/heteroaryl rings. One of these compounds, tiagabine, which is (R)-1-[4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid (**141**) is a highly potent GABA reuptake inhibitor that warranted further evaluation as an anticonvulsant agent. These important results prompted us to investigate the 4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl



(141)

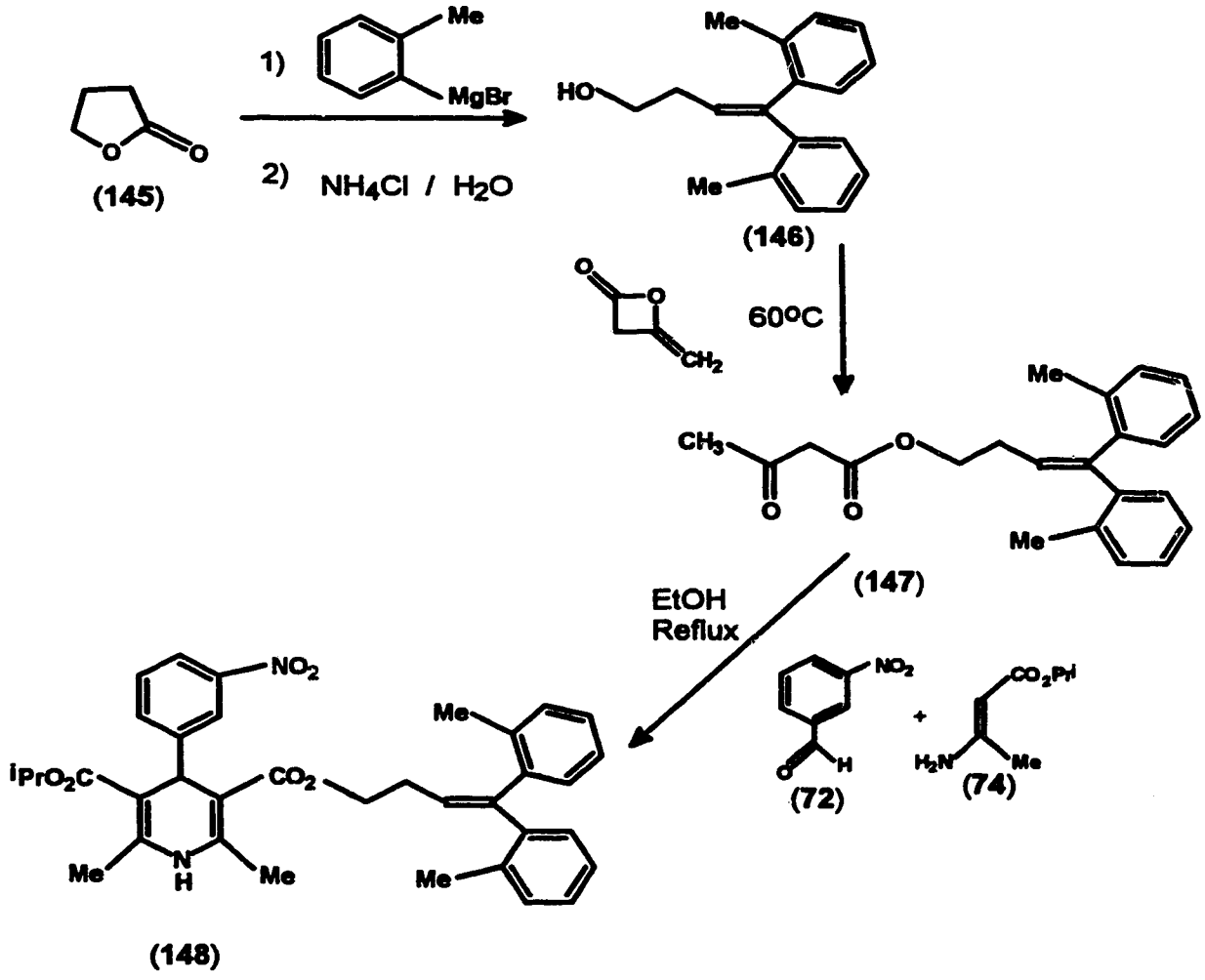
and 4,4-*bis*-(2-tolyl)-3-butenyl substituents as a method to enhance the delivery of the nimodipine analogs (144, 148) to the brain, and to determine their anticonvulsant activity.



Scheme 13. Synthesis of 3-[4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (144).

4-Bromo-1,1-*bis*-(3-methyl-2-thienyl)-1-butene (143) was synthesized according to the method suggested by Knutsen *et al.*[211] The 3-[4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl] ester DHP (144) was then prepared by the reaction of (143) with isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (142) at 25°C in the presence of potassium carbonate in DMF for 5 days (Scheme 13). When the reaction was stopped, acidification with dilute acid resulted in precipitation of the unreacted DHP (142). Purification by silica gel column chromatography provided (143) together with the product (144, 27%). The low yield in this reaction is probably due to the steric

effect of the two bulky *ortho*-methylthiophene groups which hinder the S_N2 reaction of the carboxylate anion. The ^1H NMR spectra for **(144)** indicated that the methyl substituents, and H-4, H-5 protons on the two thiophene rings were not chemically equivalent. These results suggest that the two heteroaryl rings are not coplanar possibly due to steric repulsion induced by the two *ortho*-methyl groups. A conformation in which there is restricted rotation of the heteroaryl/aryl rings may account for the increase in GABA-reuptake inhibition for *ortho*-substituted relative to non-substituted derivatives.[211]



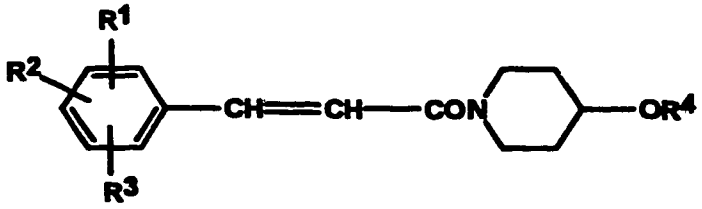
Scheme 14. Synthesis of 3-[4,4-*bis*-(*o*-tolyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (148).

The synthesis of (148) is shown in Scheme 14. Thus, reaction of two equivalents of *o*-tolylmagnesium bromide with 4-butyrolactone (145) in ether at 25^{0}C for 2 hours followed by quenching with ammonium chloride yielded 4,4-*bis*-(*o*-tolyl)-3-butenol (146, 73%) which on reaction with diketene gave 4,4-*bis*-(*o*-tolyl)-3-butenyl acetoacetate (147, 84%). Condensation of (147) with 3-nitrobenzaldehyde (72) and isopropyl 3-aminocrotonate (74) gave the product (148) in 49% yield. Once again, as in the case of (144), ^{1}H NMR spectrometry showed that the two *o*-tolyl methyl protons were non-equivalent.

3.1.9.0.0.0. Syntheses of 3-(2-methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (150, 152, 154) and 3,5-diisopropyl 1,4-dihydro-2-6-dimethyl-4-(substituted-phenyl)-3,5-pyridine-dicarboxylates (151, 153, 155)

This title class of compounds was synthesized to acquire structure-activity relationship correlations for 3-NMe_2, 4-NMe_2 and 3,4,5-trimethoxy substituents on the C-4 phenyl ring of DHP CCAs. The two 1,4-DHP ring systems selected for this study were the 5-isopropyl 3-methoxyethyl 1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate moiety present in nimodipine and the related symmetrical 3,5-diisopropyl diester.

Compounds (154) and (155) which have a C-4 3,4,5-trimethoxyphenyl substituent were selected for study based on the fact that the CCAs verapamil (31) and D-600 possess methoxyphenyl substituents. It was therefore of interest to determine whether a C-4 3,4,5-trimethoxyphenyl substituent provided good CCA activity. Compounds of (149), some of which contain an alkoxyphenyl moiety, have been reported[212] to exhibit anticonvulsive, tranquilizing and sedative activities.

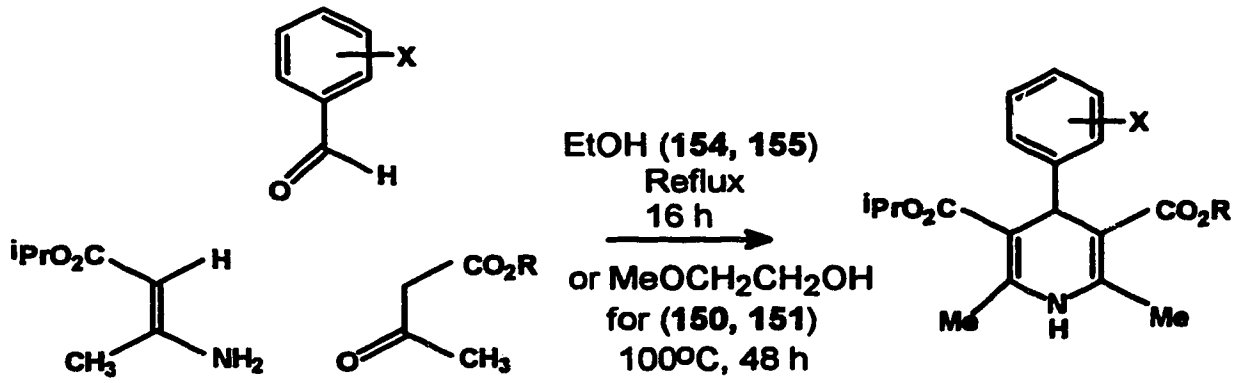


R^1, R^2, R^3 = H, OH, OMe. Alkyl, CF_3
R^4 = Alkyl

(149)

It was anticipated that compounds possessing the 3,4,5-trimethoxyphenyl substituent would undergo enhanced uptake into the brain.

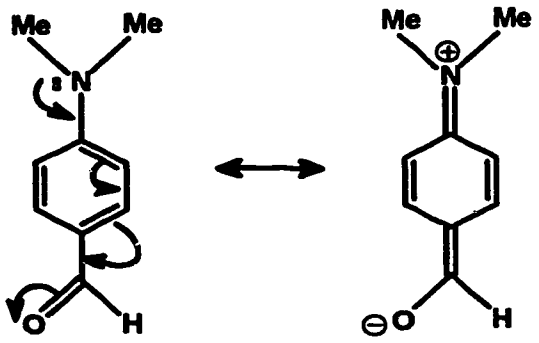
Compounds having a C-4 3- or 4-dimethylaminophenyl substituent were of interest since the dimethylamino group should undergo protonation at physiological pH to provide a cationic site which could bind to an anionic site on the DHP receptor. Since substituents at the *para*-phenyl position usually exhibit lower CCA activity (1.7.0.0.0.0.), compounds possessing 4-dimethylamino (**150, 151**) and 3-dimethylamino (**152, 153**) aryl substituents were prepared for comparison.



(150) X = 4-NMe_2 R = CH_2CH_2OMe
(151) X = 4-NMe_2 R = ^iPr
(154) X = 3,4,5-$(OMe)_3$ R = CH_2CH_2OMe
(155) X = 3,4,5-$(OMe)_3$ R = ^iPr

Scheme 15. Syntheses of 4-dimethylaminophenyl and 3,4,5-trimethoxyphenyl dihydropyridine analogs (150), (151), (154) and (155).

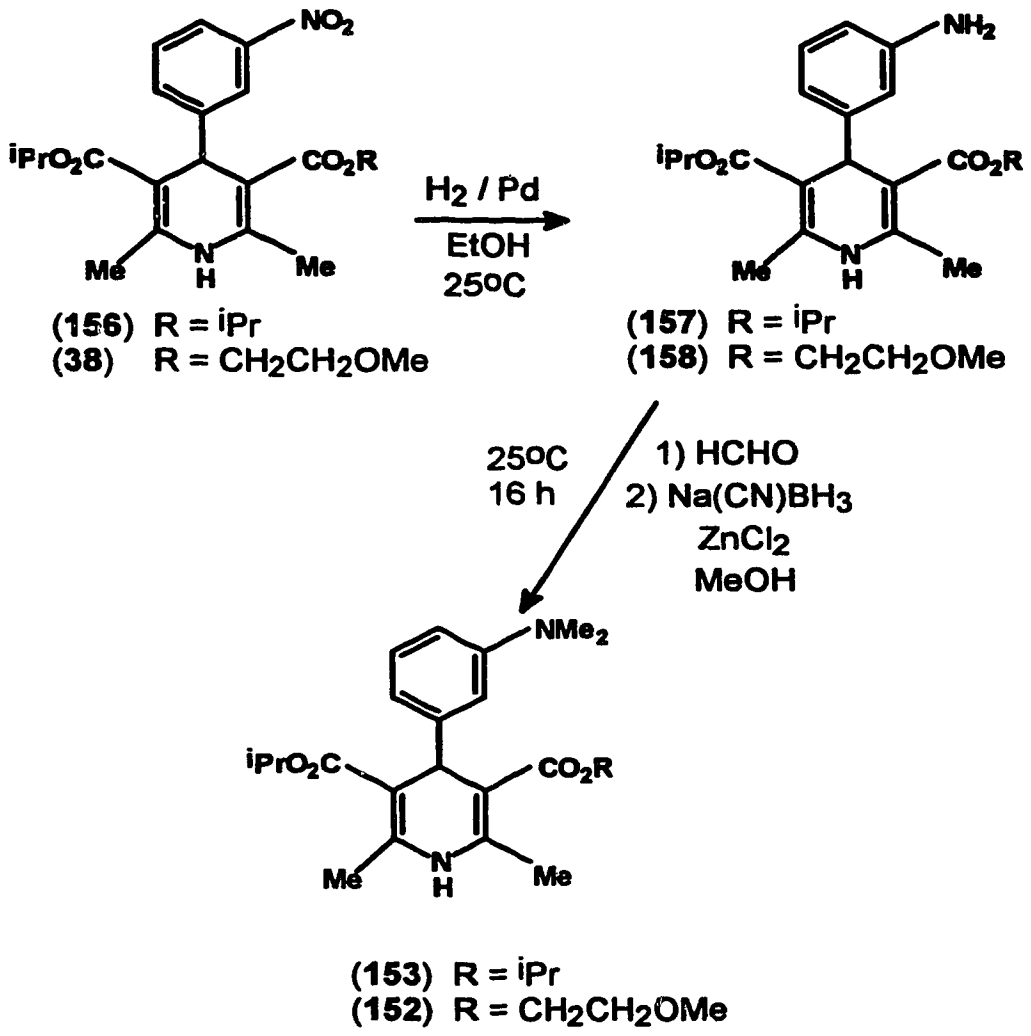
Compounds (**150, 151, 154, 155**) were prepared using a Hantzsch reaction which involved the condensation of 3,4,5-trimethoxybenzaldehyde or 4-dimethylamino-benzaldehyde, an alkyl acetoacetate and isopropyl 3-aminocrotonate, as shown in Scheme 15. This Hantzsch reaction was not successful for the synthesis of the 4-dimethylaminophenyl derivatives (**150, 151**) since no reaction occurred using the usual reaction conditions of heating at reflux temperature using ethanol as the solvent. This lack of reactivity is attributed to the fact that the dimethylamino nitrogen lone-pair of electrons is delocalized into the phenyl ring to provide conjugation with the aldehyde carbonyl group as shown in Equation 25. Consequently, the aldehyde group is less susceptible to nucleophilic attack in the Hantzsch reaction. When dioxane, which has a boiling point of 105°C was used as solvent, side-products were formed which suggests that a more polar solvent is required for the Hantzsch synthesis. Accordingly, when the reaction was carried out at 100°C using 2-methoxyethanol as solvent for 48 hours, compounds (**150**) and (**151**) were obtained in 25% and 19% yield, respectively.



(Eq. 25)

The 3-dimethylaminophenyl derivatives (**152, 153**) were synthesized using an alternate procedure (Scheme 16) since 3-dimethylaminobenzaldehyde is not commercially available. Hydrogenation of nimodipine (**38**) and 3,5-diisopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (**156**) using 10% palladium-on-carbon and hydrogen gas at 55 psi afforded the corresponding 3-aminophenyl analogs (**158**, 95%) and (**157**, 99%), respectively. The fact that neither the enamine C=C double bond nor the carbonyl ester of (**38, 156**) were hydrogenated is due

to conjugation of the enamine nitrogen with the carbonyl group. Delocalization of the nitrogen free electron pair deactivates the double bonds towards hydrogenation as shown in Equation 26 (1.6.2.3.0.0.).

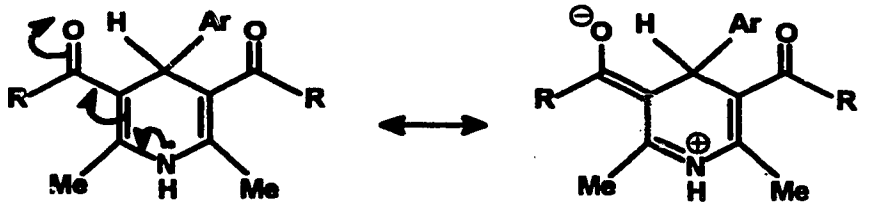


(156) R = iPr
(38) R = CH_2CH_2OMe

(157) R = iPr
(158) R = CH_2CH_2OMe

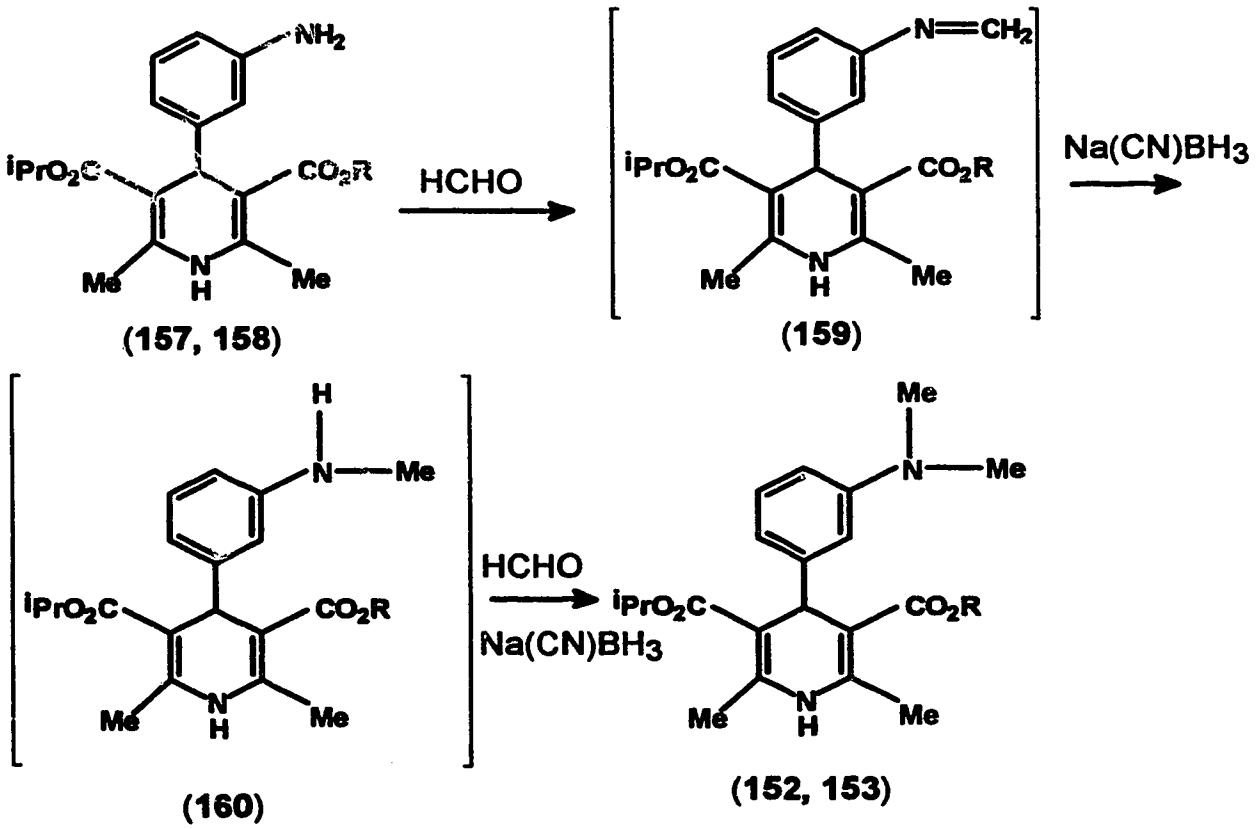
(153) R = iPr
(152) R = CH_2CH_2OMe

Scheme 16. Syntheses of 3-(2-methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-dimethylaminophenyl)-3,5-pyridinedicarboxylate (**152**) and 3,5-diisopropyl 1,4-dihydro-2,6-dimethyl-4-(3-dimethylaminophenyl)-3,5-pyridinedicarboxylate (**153**).



(Eq. 26)

The 3-aminophenyl compounds (**157, 158**), upon reaction with formaldehyde and sodium cyanoborohydride in the presence of zinc chloride at 25°C in methanol for 18 hours afforded the corresponding 3-dimethylaminophenyl products (**152**, 44%) and (**153**, 50.5%) (Scheme 16).



Scheme 17. Mechanism for the elaboration of the amino substituent to a dimethylamino substituent.

This reductive-amination, which is a modification of the Eschweiler-Clarke reaction[213], probably proceeds via an imine intermediate (**159**) which is then reduced by sodium cyanoborohydride to the methylamino intermediate (**160**). Repetition of this reaction would yield the dimethylamino products (**152, 153**) (Scheme 17). Sodium cyanoborohydride is a better reagent than sodium borohydride for this reaction since it is less reactive. The electron-withdrawing cyano-group present in sodium cyanoborohydride reduces its reactivity[214] thereby preventing the undesired reduction of the DHP ester moiety.

3.2.0.0.0.0. PHARMACOLOGICAL SCREENS

3.2.1.0.0.0. Anticonvulsant assay

The Epilepsy Division of the U.S. National Institute of Neurological and Communicative Disorders and Stroke established a standardized screening project in 1975[215] to encourage medicinal chemists and the pharmaceutical industry to develop new anticonvulsant drugs. In principle, this program consists of three successive screening procedures whose objective is to identify, quantitate and evaluate new potential anticonvulsants, respectively.

The compounds synthesized in this thesis research project were therefore evaluated by the Epilepsy Branch at the National Institute of Health in the U.S. as anticonvulsant agents. Most of the screening tests conducted on our compounds stopped at the stage I screen, although further anticonvulsant evaluation of a few compounds proceeded to the stage II screen. The results obtained provide structure-activity relationship correlations for the DHP class of compounds and some prodrugs thereof.

Although five testing methods[216] can be used for anticonvulsant screening, the two most widely used tests that were employed in this project are the Maximal Electroshock Seizure (MES) test and the Subcutaneous Pentylenetetrazole (Metrazol®) Seizure Threshold Test (scMet.).

In the MES assay, the seizure is elicited electrically by a 60 Hz current of 50 mA for 0.2 second to mice through a corneal electrode. The animal is usually challenged at 30 minutes and one hour after drug administration. Seizures are displayed as a short period of tonic flexion followed by a lengthy tonic extension of the limbs. Abolition of the tonic hind limb extension after drug treatment is accepted as seizure protection. A drug which provides protection in the MES test is regarded as effective for the treatment of generalized (grand mal) and partial seizures.[217]

In contrast, subcutaneous injection of the chemical convulsant agent pentylenetetrazole (Metrazol®) at a dosage of 85 mg/kg to mice is used to produce seizures in the scMet screen. Like the MES, the animals are challenged at 30 minutes and one hour after drug administration. The absence of seizures of a single 5 second episode during the 30 minute observation period is regarded as protection. Drugs which protect against scMet are likely to be effective against absence (petit mal) seizures.[217]

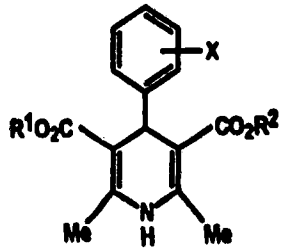
The toxicity of a compound can be determined by a number of test methods. The method employed in this project was the rotorod toxicity test where mice are observed at 30 minutes and one hour after drug administration by setting them on a rod which rotates at a speed of 6 rpm. At this speed, a normal mouse is able to remain on the rod, whereas an intoxicated mouse is unable to grasp the rod for three consecutive 1 minute trials.

3.2.2.0.0.0. Calcium channel antagonist activity

In his review of calcium channel blockers and their actions, Rahwan[218] introduced criteria and testing methods for classifying a drug as an extracellular or intracellular CCA. Although a large number of classification methods are available, most are not satisfactory due to the fact that the calcium source is not certain, or that the method employed is cumbersome. However, one of the methods which monitors the ability of a compound to antagonize potassium-induced contraction of smooth muscle is promising since only the slow-component of the calcium current is involved in such a depolarization. This ensures that the L-type calcium channel is involved. In this research project, CCA activity was determined as the molar concentration of the test compound which produced a 50% inhibition ($IC_{50} \pm SEM$) of the muscarinic receptor-mediated

(carbachol, 1.6×10^{-7} M) Ca^{2+}-dependent contraction of a guinea pig ileal longitudinal smooth muscle (GPILSM) from the dose-response curve. The use of a muscarinic agent such as carbachol is convenient, and the results obtained upon antagonism of this contraction were shown to be consistent with those obtained from inhibition of potassium-induced depolarization.[219]

Table 2. Calcium channel antagonist activity and partition coefficients for the reference drugs nimodipine (**38**) and felodipine (**39**)



Entry	X	R^1	R^2	IC_{50} (M) [a]	Partition Coefficient (Kp) [b]
38	3-NO_2	$CH(CH_3)_2$	CH_2CH_2OMe	$1.49 \pm 0.08 \times 10^{-8}$	187
39	2,3-Cl_2	CH_2CH_3	CH_3	$1.45 \pm 0.05 \times 10^{-9}$	442

Nifedipine, $IC_{50} = 1.4 \pm 0.19 \times 10^{-8}$ M [219]

[a] Calcium channel antagonist activity was determined as the molar concentration of the compound which produced a 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.6×10^{-7} M) Ca^{2+}-dependent contraction of guniea pig ileal longitudinal smooth muscle.

[b] The partition coefficient is defined as the concentration of the compound in *n*-octanol / concentration in an aqueous buffer at pH = 7.4

Table 3. Anticonvulsant test results for the reference drugs nimodipine (**38**) and felodipine (**39**).

Entry	MES (mice) [a]				scMet (mice) [b]				Toxicity Test						Class[c]
	100 mg/kg		300 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg		
	0.5 h[d]	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
38	0/3	1/3	0/1	1/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	2/2	1
39	2/3	2/3	1/1	-	0/1	0/1	0/1	-	0/4	0/2	0/8	4/4	4/4	2*/2	1

[a, b] The results for the MES and scMet seizure tests are expressed as the number of animals protected / the number of animals tested. The vehicle for the compounds was either polyethylene glycol (PEG) or methylcellulose (0.5%). The route of drug administration was by ip injection.

[c] Classification of antiepileptic results; Class 1 = anticonvulsant activity at a dose of 100 mg/kg or less, Class 2 = anticonvulsant activity at a dose greater than 100 mg/kg, Class 3 = no anticonvulsant activity up to a dose of 300 mg/kg. Class 4 = compound shows toxicity at a dose equal to or less than 30 mg/kg.

[d] Time after drug administration. * Animals died.

3.2.3.0.0.0. Calcium channel antagonist and anticonvulsant activities for nimodipine (38) and felodipine (39)

Examination of the IC_{50} values in Table 2 indicates that nimodipine (38) ($1.49 \pm 0.08 \times 10^{-8}$ M) exhibits similar CCA activity to that of the prototype DHP, nifedipine ($1.4 \pm 0.19 \times 10^{-8}$ M), while felodipine (39) ($1.45 \pm 0.05 \times 10^{-9}$ M) is ten-fold more potent. Apart from this, the more than two-fold difference in Kp values for felodipine (Kp = 442) relative to that of nimodipine (Kp = 187) indicates that felodipine is more lipophilic which should allow it to enter the brain readily.

Anticonvulsant screening test results were acquired for these two prototype DHPs (38, 39) and the test results are listed in Table 3. Both nimodipine (38) and felodipine (39) protected mice in the MES test, although the latter compound was more potent at a 100 mg/kg ip dose. Whether this observation is due to the greater CCA activity and the higher lipophilicity of (39) is not known. These two factors should be determinants of anticonvulsant activity. However, neither compound protected mice in scMet test.

A similar study by Brodie *et al*[178] reported the dose-response relationship for nimodipine in the mouse MES seizure screen. In this study, groups of 10 mice that received a 75 mg/kg ip dose showed the highest degree of protection (> 80%) at 0.5 - 2 hours after drug administration. A separate study indicated that 60% of mice were protected using a 100 mg/kg ip dose at 1 hour. In contrast, no protection was observed at the 30 minute time period, although 1 out of 3 mice (33%) was protected at 4 hours post drug administration. The contrasting results obtained in the NIH screen can be explained on the grounds that the small number of mice (1 - 4) used does not provide a valid statistical number. The effective dosage which protected 50% of mice (ED_{50} = 162 mg/kg) was subsequently determined in the Phase 2 screen for nimodipine, a value that is about twice that for the reported study (ED_{50} = 87 mg/kg).[178] The reason for this discrepancy is not known.

Although many reports[96, 97, 179] show that nimodipine is effective in the scMet seizure test, it was ineffective in the NIH screen, likely due to the small number of mice used (1 for each test).

Hypotension arising from a felodipine overdose[220] is probably due to a sudden decrease in blood pressure caused by selective vasodilation. As a consequence, baroreflex-mediated sympathetic nerve activity may then cause tachycardia which can contribute an additional side effect. In fact, an *in vivo* study using felodipine (3.5.0.0.0.0.) at doses as low as 3.5 mg/kg given by tail-vein injection is fatal to most rats.

3.2.4.0.0.0 Calcium channel antagonist and anticonvulsant activities of 3-(2-*n*-propylpentanoyloxyalkyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (98 -101)

This group of compounds (98 - 101) was designed with a valproate ester side-chain at the C-3 position to determine whether valproic acid that could be liberated upon hydrolysis would provide seizure protection in animals. In this study, the chain length of the C-3 ester alkyl chain (n = 1 - 3) was varied (98 - 101). The results for the two 3-(2-hydroxyalkyl) analogs (102, 103) are listed for comparison (Table 4, 5).

Comparison of the CCA IC_{50} values in Table 4 for the valproate prodrugs (98 - 101) with their parent compounds (102, 103) indicates a decrease in CCA activity by one order of magnitude for most compounds except for (99). If the decrease in activity is a result of a steric effect that decreases binding to the calcium channel receptor by the bulky valporate ester, the slight increase in activity (2.5 fold) exhibited by (99) would contradict this assumption. It is not known why a C-5 methyl ester increases the activity whereas the isopropyl ester decreases CCA activity. However, comparison of the IC_{50} values of (98), (100) and (101) shows that the chain length (n) may be a determinant of CCA activity where the potency order is n = 1 > 3 > 2.

Another significant observation, as shown in Table 4, is that incorporation of a valproate ester moiety increases the Kp values. This would suggest that a valproate prodrug would enhance the brain entry of DHP-valproate prodrugs.

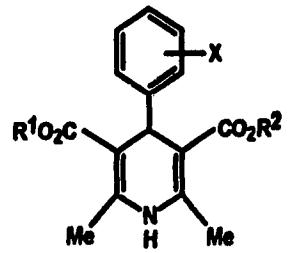
The anticonvulsant test results (Table 5), show that the single carbon spacer compound (98) was inactive in the MES and scMet seizure tests. Due to its high

toxicity, it is classified as a class 4 compound. The nature of this high toxicity is not known as toxicity is also observed for the two-carbon spacer analog (**100**).

The 2 carbon-spacer compounds (**99**) and (**100**), were active in the MES test, but not in the scMet test. The activity of the methyl ester (**99**) and the isopropyl ester (**100**) in the MES screen were qualitatively similar. However, unlike the isopropyl ester (**100**), the methyl ester (**99**) is devoid of toxic side effects. A similar finding was true for (**99**), when compared with its parent compound (**102**). Since (**99**) is a valproate prodrug of felodipine (**39**), it is appropriate to compare their activities. Both of the latter compounds exhibited considerable anticonvulsant activity in the MES screen. However, the valproate prodrug (**99**) is non-toxic indicating the valproate moiety appears to reduce toxicity of felodipine. It is not known for certain whether the MES protection is due to the DHP (**102, 103**) or the liberated valproate. Examination of their ineffectiveness (**99, 100**) in the scMet test indicates that the DHP (**102, 103**) may have provided most of the protection since valproate provides protection in both MES and scMet tests.[221]

The observation that compound (**101**) is not active suggests that a 3-carbon unit in the ester side-chain of the DHP is unfavorable for anticonvulsant activity. This may be due to an unfavorable spacial distance for a 3-carbon ester with respect to the binding to the receptor. On the other hand, the CCA activity for (**101**), reflected by its IC_{50} value, is comparable to the active analog (**100**) suggesting that anticonvulsant activity may not be due entirely to CCA activity alone.[222]

Table 4. Calcium channel antagonist activity and partition coefficients for 3-(2-*n*-propylpentanoyloxyalkyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (**98 - 101**) and 3-(2-hydroxyethyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (**102 - 103**).



Entry	X	R^1	R^2	IC_{50} (M) [a]	Partition Coefficient (Kp)[b]
98	2,3-Cl_2	$CH(CH_3)_2$	$CH_2O_2CCH(CH_2CH_2CH_3)_2$	$4.83 \pm 0.13 \times 10^{-7}$	149
99	2,3-Cl_2	CH_3	$CH_2CH_2O_2CCH(CH_2CH_2CH_3)_2$	$1.35 \pm 0.01 \times 10^{-8}$	323
100	2,3-Cl_2	$CH(CH_3)_2$	$CH_2CH_2O_2CCH(CH_2CH_2CH_3)_2$	$7.57 \pm 0.29 \times 10^{-7}$	334
101	2,3-Cl_2	$CH(CH_3)_2$	$CH_2CH_2CH_2O_2CCH(CH_2CH_2CH_3)_2$	$6.28 \pm 0.23 \times 10^{-7}$	402
102	2,3-Cl_2	CH_3	CH_2CH_2OH	$3.04 \pm 0.45 \times 10^{-8}$	236
103	2,3-Cl_2	$CH(CH_3)_2$	CH_2CH_2OH	$1.43 \pm 0.02 \times 10^{-8}$	254
Nifedipine	2-NO_2	CH_3	CH_3	$1.40 \pm 0.19 \times 10^{-8}$	-

[a] Calcium channel antagonist activity was determined as the molar concentration of the compound which produced a 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.6×10^{-7} M) Ca^{2+}-dependent contraction of guinea pig ileal longitudinal smooth muscle.

[b] The partition coefficient is defined as the concentration of the compound in *n*-octanol / concentration in an aqueous buffer at pH = 7.4

Table 5. Anticonvulsant test results for 3-(2-*n*-propylpentanoyloxyalkyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (**98 - 101**) and 3-(2-hydroxyethyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (**102 - 103**).

Entry	MES (mice) [a]				scMet (mice) [b]				Toxicity Test						Class[c]
	100 mg/kg		300 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg		
	0.5 h[d]	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
98	0/3	-	0/1	-	0/1	-	1*/1	-	0/4	2*/2	0/8	4*/4	4/4	2*/2	4
99	0/3	1/3	1/1	1/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	1
100	0/3	2/3	1/1	-	0/1	0/1	0/1	0/1	0/4	0/2	0/8	4/4	0/4	2*/2	1
101	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
102	2/2	-	-	-	1*/1	0/1	-	-	0/4	0/2	8*/8	0/1	4*/4	-	1
103	0/3	1/3	0/1	1/1	0/1	0/1	0/1	1/5	0/4	0/2	0/8	0/4	0/4	0/2	1

[a, b] The results for the MES and scMet seizure tests are expressed as the number of animals protected / the number of animals tested. The vehicle for the compounds was either polyethylene glycol (PEG) or methylcellulose (0.5%). The route of drug administration was by ip injection.

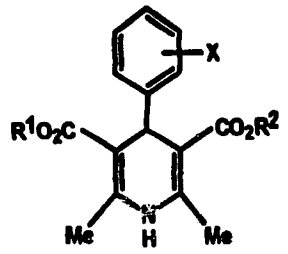
[c] Classification of antiepileptic results; Class 1 = anticonvulsant activity at a dose of 100 mg/kg or less, Class 2 = anticonvulsant activity at a dose greater than 100 mg/kg, Class 3 = no anticonvulsant activity up to a dose of 300 mg/kg. Class 4 = compound shows toxicity at a dose equal to or less than 30 mg/kg.

[d] Time after drug administration. * Animals died.

3.2.5.0.0.0. Calcium channel antagonist and anticonvulsant activities for 3-pentanoyloxyethyl 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (108 - 109).

In this series of compounds, a valerate ester was coupled to the DHP. The valerate esters (**108, 109**) exhibit significantly more potent *in vitro* CCA activity compared with their corresponding hydroxyethyl parent compounds (**102, 103**) by one to two orders of magnitude (see Table 6). Similar to the valproate prodrugs described in Table 4, the methyl ester (**108**) is a more potent CCA than the isopropyl ester (**109**) where (**108**) is almost 30 times more potent than the reference drug, nifedipine. This indicates the valerate group may be a suitable prodrug moiety with respect to CCA activity of DHPs. Although their lipophilicity, as reflected by the Kp values, is not significantly improved by the valerate ester, compounds (**108**) and (**109**) should be able to cross the BBB as they have a Kp value greater than the optimum Kp value of 100 indicated earlier (3.1.4.0.0.0.). However, the anticonvulsant effect observed does not parallel the potent CCA activity observed (Table 7). For example, compound (**108**) exhibited anticonvulsant activity only at a high dose (300 mg/kg) and a slow onset of action (3 - 4 hours) in the MES and scMet tests. This delayed onset of action could be due to a slow rate of hydrolysis of the valerate ester to release the presumably active DHP compound (**102**). This explanation is consistent with the observation that (**102**) exhibited activity in the MES screen at 30 mins following a 100 mg/kg (ip) dose.

Table 6. Calcium channel antagonist activity and partition coefficients for 3-pentanoyloxyethyl 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (**108 - 109**) and 3-(2-hydroxyethyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (**102 - 103**).



Entry	X	R^1	R^2	IC_{50} (M) [a]	Partition Coefficient (Kp) [b]
108	2,3-Cl_2	CH_3	$CH_2CH_2O_2C(CH_2)_3CH_3$	$4.53 \pm 0.21 \times 10^{-10}$	265
109	2,3-Cl_2	$CH(CH_3)_2$	$CH_2CH_2O_2C(CH_2)_3CH_3$	$3.33 \pm 0.07 \times 10^{-9}$	265
102	2,3-Cl_2	CH_3	CH_2CH_2OH	$3.04 \pm 0.45 \times 10^{-8}$	236
103	2,3-Cl_2	$CH(CH_3)_2$	CH_2CH_2OH	$1.43 \pm 0.02 \times 10^{-8}$	254

Nifedipine, $IC_{50} = 1.4 \pm 0.19 \times 10^{-8}$ M [219]
Nimodipine, $IC_{50} = 1.49 \pm 0.08 \times 10^{-8}$ M
Felodipine, $IC_{50} = 1.45 \pm 0.05 \times 10^{-9}$ M

[a] Calcium channel antagonist activity was determined as the molar concentration of the compound which produced a 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.6×10^{-7} M) Ca^{2+}-dependent contraction of guinea pig ileal longitudinal smooth muscle.

[b] The partition coefficient is defined as the concentration of the compound in *n*-octanol / concentration in an aqueous buffer at pH = 7.4

Table 7. Anticonvulsant test results for 3-pentanoyloxyethyl 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (**108 - 109**) and 3-(2-hydroxyethyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (**102 - 103**).

Entry	MES (mice) [a]				scMet (mice) [b]				Toxicity Test						Class[c]
	100 mg/kg		300 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg		
	0.5 h[d]	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
108	0/3	0/3 2/2(3 h)	0/1	1/1	0/1	0/1	0/1	3/5	0/4	0/2	0/8	0/4	0/4	0/2	2
109	0/3	1/3	0/1	-	0/1	0/1	0/1	-	0/4	0/2	0/8	0/4	4/4	2[e]/2	1
102	2/2	-	-	-	1[e]/1	0/1	-	-	0/4	0/2	8[e]/8	0/1	4[e]/4	-	1
103	0/3	1/3	0/1	1/1	0/1	0/1	0/1	1/5	0/4	0/2	0/8	0/4	0/4	0/2	1

[a, b] The results for the MES and scMet seizure tests are expressed as the number of animals protected / the number of animals tested. The vehicle for the compounds was either polyethylene glycol (PEG) or methylcellulose (0.5%). The route of drug administration was by ip injection.

[c] Classification of antiepileptic results; Class 1 = anticonvulsant activity at a dose of 100 mg/kg or less, Class 2 = anticonvulsant activity at a dose greater than 100 mg/kg, Class 3 = no anticonvulsant activity up to a dose of 300 mg/kg. Class 4 = compound shows toxicity at a dose equal to or less than 30 mg/kg.

[d] Time after drug administration. [e] Animals died.

3.2.6.0.0.0. Calcium channel antagonist and anticonvulsant activities for 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-alkyl 1,4-dihydro-2,6- dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (118 - 120) and 3-ethyl 5-methyl 1,4-dihydro-2-[2-[(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethoxy] methyl]-6-methyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (131)

In this series of compounds, selected DHPs were coupled to a brain-selective 1-methyl-1,4-dihydropyridyl-3-carbonyloxy CDS. It was anticipated that the Bodor CDS would enhance brain-uptake of the DHP-CDS compounds, which could then undergo oxidation to the pyridinium salt that would be "locked-in" the brain. Subsequent hydrolysis of the pyridinium salt ester moiety could then liberate the DHP drug to exert its anticonvulsant effect. The CCA and anticonvulsant test results for the DHP-CDS compounds (**118, 119, 120 and 131**), relative to the hydroxyethyl DHP compounds (**102, 110, 111 and 128**), are presented in Tables 8 and 9.

Comparison of the results for (**118 - 120**) and (**131**) with their corresponding hydroxyethyl DHP compounds (**102, 110, 111**) and (**128**) reveals that this CDS increases the Kp values of all DHPs by more than a hundred (Table 8). This suggests that the CDS would enhance their brain uptake. In contrast, comparison of the CCA IC_{50} values reflects a minimal effect on CCA activity due to the CDS since (**118**) and (**119**) are not affected, whereas there is a 3-fold increase in activity for (**120**). On the other hand, the amlodipine analog (**131**) exhibits a 4.5-fold decrease in CCA activity. Examination of the IC_{50} values for compounds (**118 - 120**) indicates similar CCA activities in this series where the potency order is 3-Br > 2,3-Cl_2 > 3-CF_3. This suggests that a bulky 3-phenyl substituent such as bromine would increase the CCA activity. However, for the 3-hydroxyethyl compounds (**102, 110, 111**), the activity profile is 2,3-Cl_2 > 3-CF_3 > 3-Br. The reason for this discrepancy with respect to the 3-phenyl substituents in the CDS compounds is not known.

The anticonvulsant test results (Table 9) showed that the DHP-CDS compounds (**120, 131**) were inactive in both the MES and scMet screens, whereas the trifluoromethylphenyl derivative (**119**) showed minimal activity (1/5) at 30 mins for a 300 mg/kg ip dose in the scMet assay. In contrast, the felodipine-CDS (**118**) and the

corresponding hydroxyethyl compound (**102**) exhibited protective activity in the MES test. The CDS (**118**) has a very slow onset of action (4 hour after drug administration) and the hydroxyethyl compound (**102**) appears to be quite toxic.

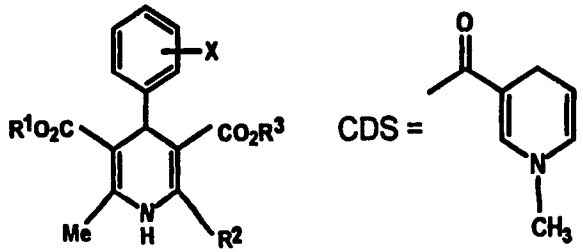
The 3-bromophenyl CDS compound (**120**) was inactive (Class 3) which is in contrast to its parent hydroxyethyl analog (**111**) (Class 2). The reverse was found for the 3-(trifluoromethylphenyl) CDS compound (**119**) (Class 2), which was marginally active, as compared to its parent compound (**110**) (Class 3) which was inactive.

The 2-hydroxyethoxymethyl amlodipine analog (**128**) was the most active CCA in this series of compounds. It offered some protection in the MES seizure assay at 4 hours for a 100 mg, or 300 mg/kg ip dose. This observation is similar to that reported for amlodipine which has a long duration of action when used as an antihypertensive agent (1.6.0.0.0.0.). One could speculate that the anticonvulsant mode of action for (**128**) is similar to the antihypertensive mode of action of amlodipine which involves blocking L-type calcium channels. In contrast, the CDS analog of amlodipine (**131**) was not active in the MES and scMet anticonvulsant tests. A similar result was obtained for the 3-bromophenyl derivatives (**120**) and (**111**) where the hydroxyethyl compound (**111**) showed some activity in the MES screen whereas the CDS-compound (**120**) was inactive. The fact that the CCA IC_{50} values for this series of compounds are comparable to that of nimodipine (Table 2) indicates that the anticonvulsant activity cannot be attributed to CCA activity alone. Since the partition coefficient values for the CDS derivatives are larger than the corresponding parent drugs (Table 8), passage across the BBB should occur readily. It was therefore of interest to investigate the reason why these CDS compounds were inactive, or that they exhibited marginal anticonvulsant activity. Several hypotheses can be used to explain the results. First of all, the CDS ester group may not undergo hydrolysis. Alternatively, if oxidation occurs rapidly in the periphery (blood) prior to the desired entry into brain, the quaternary pyridinium salt produced would be rapidly cleared from the periphery and it would not be able to cross the BBB. In contrast, if oxidation of the CDS is slow in brain, then rapid egresion of the CDS-compound from the brain would result in a short duration of action or inactivity in the anticonvulsant tests. A slow hydrolysis rate of the ester prodrug in brain could also

be an explanation for weak anticonvulsant activity. For example, it was reported in a review[223] that in order to improve the GI oral absorption of β-lactams, simple ester prodrugs, such as methyl or benzyl esters of β-lactams were not hydrolysed *in vivo* by esterases in some animals. This was probably due to a steric effect induced by the bulky β-lactam moiety with respect to interaction with the enzyme.

In vitro incubation and *in vivo* distribution studies for the felodipine-CDS (**118**) were therefore carried out to acquire information which could be used to explain the modest, or lack of, anticonvulsant activity observed for this class of CDS-compounds. The objective of the *in vitro* study was to determine the stability of the CDS (**118**) in both rat plasma and brain homogenate. *In vivo* administration of (**118**) to rats and subsequent HPLC quantitation of the CDS-drug, oxidation and hydrolysis products would provide valuable information regarding brain-uptake, including the rate of oxidation and hydrolysis in both the CNS and the periphery. It was envisaged that these results would offer explanations for the anticonvulant test results described previously.

Table 8. Calcium channel activity and partition coefficients for 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (**118 - 120**) and 3-ethyl 5-methyl 1,4-dihydro-2-[2-[(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethoxy]methyl]-6-methyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**131**) and their corresponding hydroxyethyl derivatives (**102, 110, 111, 128**).



Entry	X	R^1	R^2	R^3	IC_{50} (M) [a]	Partition Coefficient (Kp)[b]
118	2,3-Cl_2	CH_3	CH_3	CH_2CH_2O(CDS)	$3.10 \pm 0.54 \times 10^{-8}$	366
119	3-CF_3	$CH(CH_3)_2$	CH_3	CH_2CH_2O(CDS)	$5.50 \pm 0.40 \times 10^{-8}$	392
120	3-Br	$CH(CH_3)_2$	CH_3	CH_2CH_2O(CDS)	$2.45 \pm 0.09 \times 10^{-8}$	452
131	2,3-Cl_2	CH_3	$CH_2OCH_2CH_2O$(CDS)	CH_2CH_3	$2.99 \pm 0.96 \times 10^{-8}$	344
102	2,3-Cl_2	CH_3	CH_3	CH_2CH_2OH	$3.04 \pm 0.45 \times 10^{-8}$	236
110	3-CF_3	$CH(CH_3)_2$	CH_3	CH_2CH_2OH	$5.39 \pm 0.20 \times 10^{-8}$	282
111	3-Br	$CH(CH_3)_2$	CH_3	CH_2CH_2OH	$7.58 \pm 0.16 \times 10^{-8}$	295
128	2,3-Cl_2	CH_3	$CH_2OCH_2CH_2OH$	CH_2CH_3	$6.56 \pm 0.48 \times 10^{-9}$	227

Nifedipine, $IC_{50} = 1.4 \pm 0.19 \times 10^{-8}$ M [219]; Nimodipine, $IC_{50} = 1.49 \pm 0.08 \times 10^{-8}$ M; Felodipine, $IC_{50} = 1.45 \pm 0.05 \times 10^{-9}$ M

[a] Calcium channel antagonist activity was determined as the molar concentration of the compound which produced a 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.6×10^{-7} M) Ca^{2+}-dependent contraction of guinea pig ileal longitudinal smooth muscle.

[b] The partition coefficient is defined as the concentration of the compound in *n*-octanol / concentration in an aqueous buffer at pH = 7.4

Table 9. Anticonvulsant test results for 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (**118 - 120**), 3-ethyl 5-methyl 1,4-dihydro-2-[2-[(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethoxy]methyl]-6-methyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**131**) and their corresponding hydroxyethyl derivatives (**102, 110, 111, 128**).

Entry	MES (mice) [a]				scMet (mice) [b]				Toxicity Test						Class[c]
	100 mg/kg		300 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg		
	0.5 h[d]	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
118	0/3	1/3	0/1	1/1	0/1	0/1	0/1	1*/1	0/4	0/2	0/8	0/4	0/4	1/2	1
119	0/3	0/3	0/1	0/1	0/1	0/1	1/5	0/1	0/4	0/2	0/8	0/4	0/4	0/2	2
120	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
131	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
102	2/2	-	-	-	1*/1	0/1	-	-	0/4	0/2	8*/8	0/1	4*/4	-	1
110	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
111	0/1	0/3	1/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	2
128	0/3	1/3	0/1	1/1	0/1	0/1	0/1	1/5	0/4	0/2	0/8	0/4	0/4	0/2	1

[a, b] The results for the MES and scMet seizure tests are expressed as the number of animals protected / the number of animals tested. The vehicle for the compounds was either polyethylene glycol (PEG) or methylcellulose (0.5%). The route of drug administration was by ip injection.

[c] Classification of antiepileptic results; Class 1 = anticonvulsant activity at a dose of 100 mg/kg or less, Class 2 = anticonvulsant activity at a dose greater than 100 mg/kg, Class 3 = no anticonvulsant activity up to a dose of 300 mg/kg. Class 4 = compound shows toxicity at a dose equal to or less than 30 mg/kg.

[d] Time after drug administration. * Animals died.

3.2.7.0.0.0. Anticonvulsant evaluation of 3-[2-[4-(4-fluorophenyl)piperazinyl]ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridine-dicarboxylate (137)

The title compound (137) exhibited CCA activity (IC_{50} = 1.59 ± 0.09 x 10^{-8} M) comparable to that of the reference drugs nifedipine (IC_{50} = 1.4 ± 0.19 x 10^{-8} M) or nimodipine (IC_{50} = 1.49 ± 0.08 x 10^{-8} M) (Table 10). Although (137) exhibited anticonvulsant activity in the qualitative scMet seizure screen at a dose of 100 mg/kg ip, its high toxicity deterred further evaluation (Table 11).

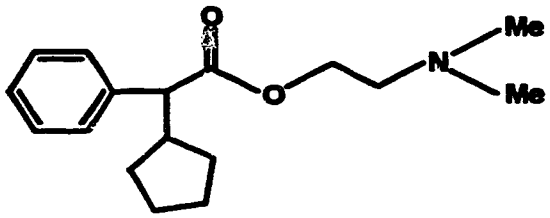
3.2.8.0.0.0. Calcium channel antagonist and anticonvulsant activities of 3-[2-(dimethylamino)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (139) and 3-[2-(trimethylammonium)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate iodide (140)

As indicated in Table 10, quaternization of the dimethylamine moiety (139) produced an almost thousand-fold decrease in CCA activity. This elaboration indicates a cationic group in the C-3 ester substituent nearly abolishes CCA activity. In addition, the very low lipophilicity (Kp = 0.15) for (140) makes it a poor candidate as a brain targeted anticonvulsant agent.

The quaternary ammonium derivative (140) was extremely toxic since it was 100% lethal at a 30 mg/kg ip dose in mice within 30 minutes (see Table 11). It is possible that this acute toxicity is caused by the acetylcholine-like C-3 ester substituent. Acetylcholine is an important cholinergic neurotransmitter that is responsible for a large number of physiological events such as movement of both voluntary and involuntary muscle, ganglionic synaptic transmission and glandular secretion.

On the other hand, the dimethylamino derivative (139) provided significant protection in the MES screen at a 100 and 300 mg/kg ip dose although the duration of action was short (30 min.). Compound (139) is therefore able to gain access to the brain, but its high lipophilicity (Kp = 230) likely results in its rapid egress from brain which would explain its short duration of action. Compound (139), like

its quaternary ammonium analog (140), is markedly toxic. Again, this toxicity may be due, at least in part, to a cholinergic effect even though it does not possess a quaternary ammonium moiety. For example, the muscarinic antagonist cyclopentolate (161) possesses a dimethylaminoethyl ester moiety similar to (139). It is therefore possible to speculate that the toxicity of (139) could be due, at least in part, to a potent CNS cholinergic agonist effect.

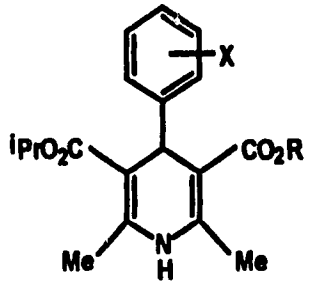


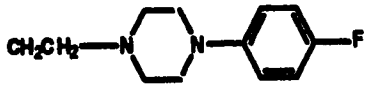
(161)

3.2.9.0.0.0. Calcium Channel antagonist and anticonvulsant activities for 3-[4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (144) and 3-[4,4-*bis*-(*o*-tolyl)]-3-butenyl 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (148)

The results presented in Tables 12 and 13 indicated that compounds (144) and (148) are both inactive in the MES and scMet anticonvulsant screens, and that they possess high partition coefficient values (Kp = 385 - 424). This high lipophilicity should provide access to the brain. Although the CCA activity of the *bis*-thienyl prodrug (144) (IC_{50} = 4.45 ± 0.28 x 10^{-6} M) is two orders of magnitude lower than nimodipine (IC_{50} = 1.49 ± 0.08 x 10^{-8} M) (Table 12), compound (148) (IC_{50} = 2.25 ± 0.01 x 10^{-8} M) that has a comparable CCA activity to nimodipine should exhibit some anticonvulsant activity if CCA activity is taken to be the sole mechanism for seizure control. The reason for the inactivity of these two compounds is not known. The bulky *bis*-aryl (heteroaryl) butene side chain may prevent their binding to the receptor site. *In vivo* animal studies and subsequent quantitative analyses of the drug in brain may provide information to explain the inactivity of compounds (144) and (148).

Table 10. Calcium channel antagonist and partition coefficients for 3-[2-[4-(4-fluorophenyl)piperazinyl]ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**137**), 3-[2-(dimethylamino)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (**139**) and 3-[2-(trimethylammonium)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (**140**).



Entry	X	R	IC_{50} (M) [a]	Partition Coefficient (Kp)[b]
137	2,3-Cl_2		$1.59 \pm 0.09 \times 10^{-8}$	265
139	3-NO_2	$CH_2CH_2N(CH_3)_2$	$6.07 \pm 0.60 \times 10^{-8}$	230
140	3-NO_2	$CH_2CH_2N(CH_3)_3^+ I^-$	$1.39 \pm 0.00 \times 10^{-5}$	0.15

Nifedipine, $IC_{50} = 1.4 \pm 0.19 \times 10^{-8}$ M [219]

Nimodipine, $IC_{50} = 1.49 \pm 0.08 \times 10^{-8}$ M;

Felodipine, $IC_{50} = 1.45 \pm 0.05 \times 10^{-9}$ M

[a] Calcium channel antagonist activity was determined as the molar concentration of the compound which produced a 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.6×10^{-7} M) Ca^{2+}-dependent contraction of guinea pig ileal longitudinal smooth muscle.

[b] The partition coefficient is defined as the concentration of the compound in *n*-octanol / concentration in an aqueous buffer at pH = 7.4

Table 11. Anticonvulsant test results for 3-[2-[4-(4-fluorophenyl)piperazinyl]ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (137), 3-[2-(dimethylamino)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (139) and 3-[2-(trimethylammonium)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate iodide (140).

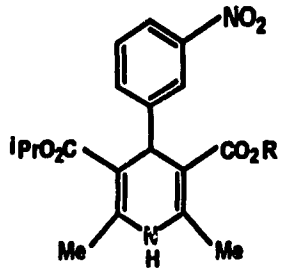
Entry	MES (mice) [a]				scMet (mice) [b]				Toxicity Test						Class[c]
	100 mg/kg		300 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg		
	0.5 h[d]	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
137	0/3	-	0/1	-	1/1	-	-	-	0/4	1*/1	8/8	4/4	4*/4	-	4
139	3/3	0/3	1/1	-	1*/1	-	-	-	0/4	0/2	7/8	3*/4	4*/4	-	1
140	-	-	-	-	-	-	-	-	4*/4	-	8*/8	-	4*/4	-	4

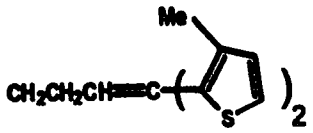
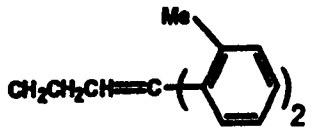
[a, b] The results for the MES and scMet seizure tests are expressed as the number of animals protected / the number of animals tested. The vehicle for the compounds was either polyethylene glycol (PEG) or methylcellulose (0.5%). The route of drug administration was by ip injection.

[c] Classification of antiepileptic results; Class 1 = anticonvulsant activity at a dose of 100 mg/kg or less, Class 2 = anticonvulsant activity at a dose greater than 100 mg/kg, Class 3 = no anticonvulsant activity up to a dose of 300 mg/kg. Class 4 = compound shows toxicity at a dose equal to or less than 30 mg/kg.

[d] Time after drug administration. * Animals died.

Table 12. Calcium channel antagonist activity and partition coefficients for 3-[4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (**144**) and 3-[4,4-*bis*-(*o*-tolyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (**148**).



Entry	R	IC_{50} (M) [a]	Partition Coefficient (Kp)[b]
144		$4.45 \pm 0.28 \times 10^{-6}$	424
148		$2.25 \pm 0.01 \times 10^{-8}$	385

Nifedipine, $IC_{50} = 1.4 \pm 0.19 \times 10^{-8}$ M [219]
Nimodipine, $IC_{50} = 1.49 \pm 0.08 \times 10^{-8}$ M
Felodipine, $IC_{50} = 1.45 \pm 0.05 \times 10^{-9}$ M

[a] Calcium channel antagonist activity was determined as the molar concentration of the compound which produced a 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.6×10^{-7} M) Ca^{2+}-dependent contraction of guinea pig ileal longitudinal smooth muscle.

[b] The partition coefficient is defined as the concentration of the compound in *n*-octanol / concentration in an aqueous buffer at pH = 7.4

Table 13. Anticonvulsant activities for 3-[4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (144) and 3-[4,4-*bis*-(*o*-tolyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (148).

Entry	MES (mice) [a]				scMet (mice) [b]				Toxicity Test						Class[c]
	100 mg/kg		300 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg		
	0.5 h[d]	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
144	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
148	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3

[a, b] The results for the MES and scMet seizure tests are expressed as the number of animals protected / the number of animals tested. The vehicle for the compounds was either polyethylene glycol (PEG) or methylcellulose (0.5%). The route of drug administration was by ip injection.

[c] Classification of antiepileptic results; Class 1 = anticonvulsant activity at a dose of 100 mg/kg or less, Class 2 = anticonvulsant activity at a dose greater than 100 mg/kg, Class 3 = no anticonvulsant activity up to a dose of 300 mg/kg. Class 4 = compound shows toxicity at a dose equal to or less than 30 mg/kg.

[d] Time after drug administration. [e] Animals died.

3.2.10.0.0.0. Calcium channel antagonist and anticonvulsant activities for 3-(2-methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (150, 152, 154) and 3,5-diisopropyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (151, 153, 155)

Examination of the CCA test results (Table 14) shows that the point of attachment, and possibly the nature, of the phenyl substituent was a determinant of potency where the activity profile was *meta* > *para* > 3,4,5-trimethoxy. In addition, the symmetrical diisopropyl derivatives (151), (153) are more potent CCAs than their corresponding unsymmetrical 3-(2-methoxyethyl) analogs (150), (152) although the reverse is true for the 3,4,5-trimethoxyphenyl analogs (154) and (155). Another significant result from Table 14 indicates that the dimethylamino substituent in (150 - 153) lowers the Kp value. This may be due to the more hydrophilic nature of the amine group. However, the Kp values of (150 - 153) are suitably lipophilic for brain delivery (optimum Kp value for brain entry is 100) (3.1.4.0.0.0.). Triggle *et al*[157, 158] compared the CCA activities for different types of DHPs, as determined by their ability to inhibit the calcium dependent contraction of guinea pig ileal smooth muscle by a muscarinic agonist, with their X-ray crystalline structure conformation. Triggle concluded that CCA activity was dependent upon the degree of DHP ring pucker, and that the most active compounds are those with the least deviation from a relatively flat boat structure. The fact that compounds possessing *ortho* and *meta*-phenyl substituents are more active than *para*-substituted analogs is due to non-bonded repulsion exerted between the *ortho*- and *meta*-phenyl substituents and the C-3 and C-5 ester substituents. As a result, the boat conformation becomes a flatter boat when interactions between the C-3 or C-5 ester and the phenyl substituent increases. A *para*-phenyl substituent offers the least steric effect and hence the boat is more puckered (less flat) and CCA activity is decreased.

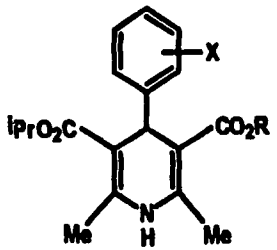
Apart from the effect of the phenyl-substituent, the orientation of the C-3 and C-5 ester moieties is also critical to CCA activity as discussed in 1.7.1.0.0.0. - 1.7.2.0.0.0. For a DHP to exert CCA activity, one or preferably, both ester moieties should have the

carbonyl group *cis* to the carbon - carbon double bond of the DHP ring so that a favorable H-bond can be formed with the receptor site (Figure 7).[135, 172]

Although the anticonvulsant activities indicate that this series of compounds are marginally active (Class 2) or inactive (Class 3), the results in Table 15 appear to indicate a similar activity order, which is *meta* > *para* > 3,4,5-trimethoxy. This is supported by the fact that both of the *meta*-phenyl derivatives (152), (153) are active (Class 2) while the *para*-phenyl derivatives show activity for the methoxyethyl analog (150), and that the 3,4,5-trimethoxyphenyl compounds (154) and (155) are devoid of activity. These results suggest that although CCA activity is not the sole factor, it plays a significant role in seizure protection.

Molecular mechanics calculations for the diisopropyl ester derivatives (151, 153, 155) and nimodipine (38) were performed using the IBM-PC Version Alchemy II minimizer program (3.3.0.0.0.0.). The calculations show no difference between active (38, 153) and inactive (151, 155) compounds with respect to ring puckering as determined by the interspatial distance between N(1) and C(4) of the DHP ring. In general, substituents at the *meta*-position(s) of the phenyl ring have little effect and a *para*-substituent has a neligible effect, on the degree of ring pucker due to their greater distance from the ester C=O oxygen moiety.

Table 14. Calcium channel antagonist activity and partition coefficients for 3-(2-methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (**150, 152, 154**) and 3,5-diisopropyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (**151, 153, 155**).



Entry	X	R	IC_{50} (M) [a]	Partition Coefficient (Kp) [b]
150	4-$N(CH_3)_2$	$CH_2CH_2OCH_3$	$1.33 \pm 0.02 \times 10^{-5}$	121
151	4-$N(CH_3)_2$	$CH(CH_3)_2$	$7.61 \pm 1.50 \times 10^{-6}$	104
152	3-$N(CH_3)_2$	$CH_2CH_2OCH_3$	$1.96 \pm 0.03 \times 10^{-7}$	137
153	3-$N(CH_3)_2$	$CH(CH_3)_2$	$3.47 \pm 0.26 \times 10^{-8}$	129
154	3,4,5-$(OCH_3)_3$	$CH_2CH_2OCH_3$	$2.84 \pm 0.00 \times 10^{-5}$	208
155	3,4,5-$(OCH_3)_3$	$CH(CH_3)_2$	$3.09 \pm 0.03 \times 10^{-5}$	259

Nifedipine, the $IC_{50} = 1.4 \pm 0.19 \times 10^{-8}$ M [219]

Nimodipine, $IC_{50} = 1.49 \pm 0.08 \times 10^{-8}$ M

Felodipine, $IC_{50} = 1.45 \pm 0.05 \times 10^{-9}$ M

[a] Calcium channel antagonist activity was determined as the molar concentration of the compound which produced a 50% inhibition of the muscarinic receptor-mediated (carbachol, 1.6×10^{-7} M) Ca^{2+}-dependent contraction of guinea pig ileal longitudinal smooth muscle.

[b] The partition coefficient is defined as the concentration of the compound in *n*-octanol / concentration in an aqueous buffer at pH = 7.4

Table 15. Anticonvulsant test results for 3-(2-methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (150, 152, 154) and 3,5-diisopropyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (151, 153, 155).

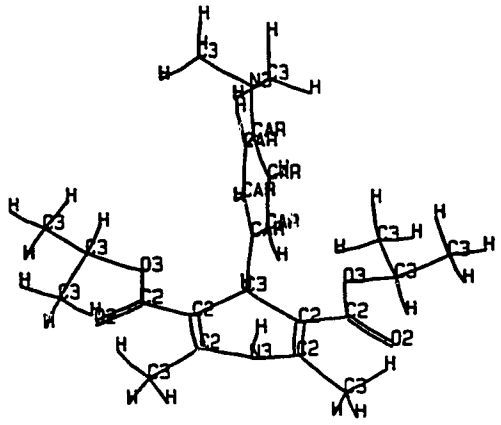
Entry	MES (mice) [a]				sc Met (mice) [b]				Toxicity Test						Class[c]
	100 mg/kg		300 mg/kg		100 mg/kg		300 mg/kg		30 mg/kg		100 mg/kg		300 mg/kg		
	0.5 h[d]	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
150	0/3	0/3	0/1	1/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	2
151	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
152	0/3 2/2 (0.25)	0/3 1/2 (1)	1/1	1/1	0/1	0/1	0/1	1/1	0/4	0/2	2/8	4/4	4/4	2/2	2
153	0/3	0/3	0/1	1/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	1/2	2
154	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3
155	0/3	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/2	0/8	0/4	0/4	0/2	3

[a, b] The results for the MES and scMet seizure tests are expressed as the number of animals protected / the number of animals tested. The vehicle for the compounds was either polyethylene glycol (PEG) or methylcellulose (0.5%). The route of drug administration was by ip injection.

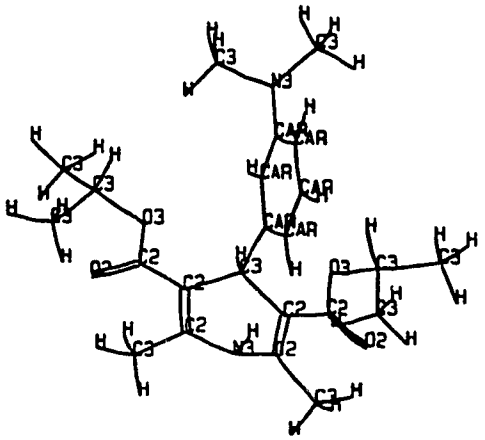
[c] Classification of antiepileptic results; Class 1 = anticonvulsant activity at a dose of 100 mg/kg or less, Class 2 = anticonvulsant activity at a dose greater than 100 mg/kg, Class 3 = no anticonvulsant activity up to a dose of 300 mg/kg. Class 4 = compound shows toxicity at a dose equal to or less than 30 mg/kg.

[d] Time after drug administration. [e] Animals died.

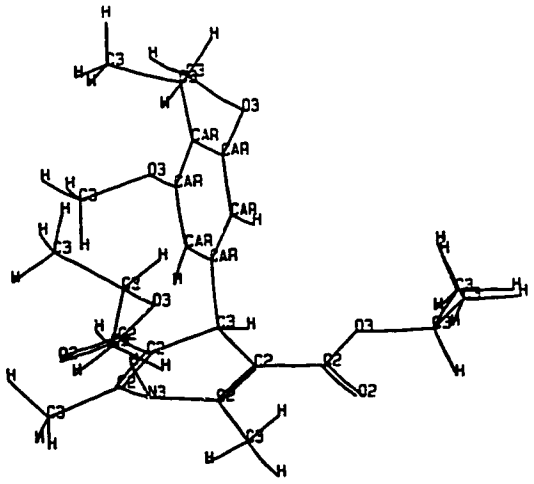
3.3.0.0.0.0. Minimized structures for 3,5-diisopropyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (151, 153, 155) and nimodipine (38).



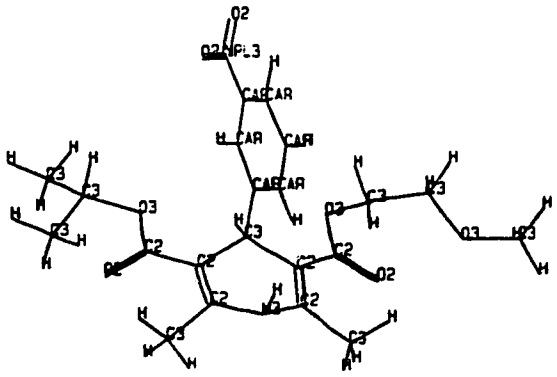
4-Dimethylaminophenyl-DHP (151)



3-Dimethylaminophenyl-DHP (153)



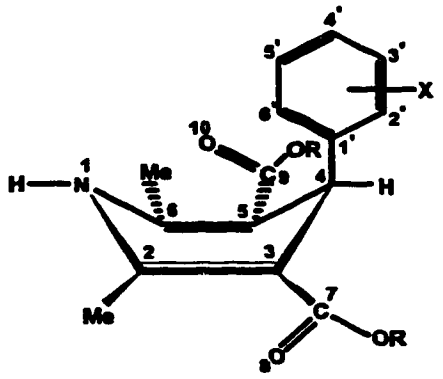
3,4,5-Trimethoxyphenyl-DHP (155)



Nimodipine (38)

Figure 9. The optimized conformations for compounds (151), (153), (155) and nimodipine (38).

Table 16. The molecular parameters, bond length (distance), bond angle and torsional angle for the DHPs (151), (153), (155) and nimodipine (38).



Compound	(151)	(153)	(155)	(38)
Interatomic distances (Å)				
N(1) - C(2)	1.335	1.336	1.335	1.334
C(2) - C(3)	1.345	1.344	1.345	1.345
C(3) - C(4)	1.510	1.511	1.510	1.509
C(4) - C(5)	1.509	1.510	1.510	1.509
C(5) - C(6)	1.345	1.347	1.346	1.345
C(6) - N(1)	1.335	1.335	1.334	1.335
N(1) - C(2')	4.875	4.848	4.865	4.872
N(1) - C(6')	3.393	3.352	3.355	3.429
N(1) - C(3')	5.851	5.881	5.827	5.881
N(1) - C(5')	4.687	4.635	4.635	4.737
N(1) - C(4)	2.746	2.746	2.749	2.736
Bond angle (°)				
C(2) - N(1) - C(6)	116.7	116.6	116.7	116.1
N(1) - C(2) - C(3)	122.2	122.2	122.3	121.9
C(2) - C(3) - C(4)	116.8	116.8	116.8	116.6
C(3) - C(4) - C(5)	111.4	111.2	111.1	110.9

	(151)	(153)	(155)	(38)
C(4) - C(5) - C(6)	116.7	116.7	116.9	116.6
C(5) - C(6) - N(1)	122.3	122.3	122.2	121.9
H(4) - C(4) -C(1')	107.6	107.8	107.9	107.2
Torsonal angle (°)				
N(1) - C(2) - C(3) - C(4)	2.5	2.2	2.2	2.6
C(2) - C(3) - C(4) - C(5)	25.6	26.4	25.8	27.3
C(3) - C(4) - C(5) - C(6)	-25.5	-26.5	-25.9	-27.2
C(4) - C(5) - C(6) - N(1)	-2.7	-1.9	-2.1	-2.8
C(6) - N(1) - C(2) - C(3)	-33.1	-33.0	-32.7	-35.2
C(6) - C(5) - C(9) - O(10)	1.2	0.0	0.9	2.7
C(2) - C(3) - C(7) - O(8)	0.0	0.0	0.0	0.0
C(2) - C(3) - C(4) - C(1')	-99.6	-98.1	-98.5	-99.8
C(6) - C(5) - C(4) - C(1')	98.4	96.6	97.3	98.8
C(5) - C(4) - C(1') - C(2')	127.7	130.3	129.1	130.7
C(3) - C(4) - C(1') - C(2')	-107.6	-105.8	-107.0	-103.5
Energy (kcal / mole)	-5.7	-4.6	-3.6	+4.9

The molecular parameters were determined using the Alchemy II program from Tripos Associates Inc., (IBM-PC Version).

The optimized conformations for **(151)**, **(153)**, **(155)** and nimodipine **(38)**, as illustrated in Figure 9, indicate that the 1,4-DHP ring exists in a rather flat boat conformation with the phenyl ring having a pseudoaxial orientation. This pseudoaxial orientation of the phenyl ring is also reflected by the near 90° torsion angle formed by C(2) - C(3) - C(4) - C(1'). The degree of 1,4-DHP ring pucker as determined by the N(1) - C(4) interspatial distance, suggests these four compounds possess a similar degree of ring puckering. The torsion angles for C(6) - C(5) - C(9) - O(10) and C(2) - C(3) - C(7) -O(8) are close to 0° which indicates that the two carbonyl groups are in the same plane and *cis* to the carbon-carbon double bonds of the DHP ring. Also, the torsion

angle formed by C(5) - C(4) - C(1') - C(2') does not deviate very much from 120^0 suggesting that the phenyl ring bisects the DHP ring. Compounds with a 3-phenyl substitutent such as (153) and (38) display an interatomic distance N(1) - C(3') that is larger that N(1) - C(6'). This indicates that the phenyl-substitutent is *syn*-periplanar to H-4 of the DHP ring.

Although little difference in molecular parameters was evident between active and inactive compounds that were examined in this study, the information acquired supports the known conformation for Hantzsch dihydropyridines.

3.4.0.0.0.0. *In Vitro* incubation studies employing felodipine (39) as the substrate

Since (118) is a felodipine-CDS compound, incubation studies using felodipine (39) as the substrate were carried out so that the results could be compared with those of the CDS compound (118). Quantitative HPLC analyses was performed using acetonitrile-water (1:1, v/v) as eluent at a flow rate of 1 ml/min using a 3.9 x 150 mm reverse phase C_{18} column with UV detection at 350 nm. Under these conditions, felodipine had a retention time (R_T) of 10 min (Fig. 10). The internal standard employed in these studies was 3-(2-hydroxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (102).

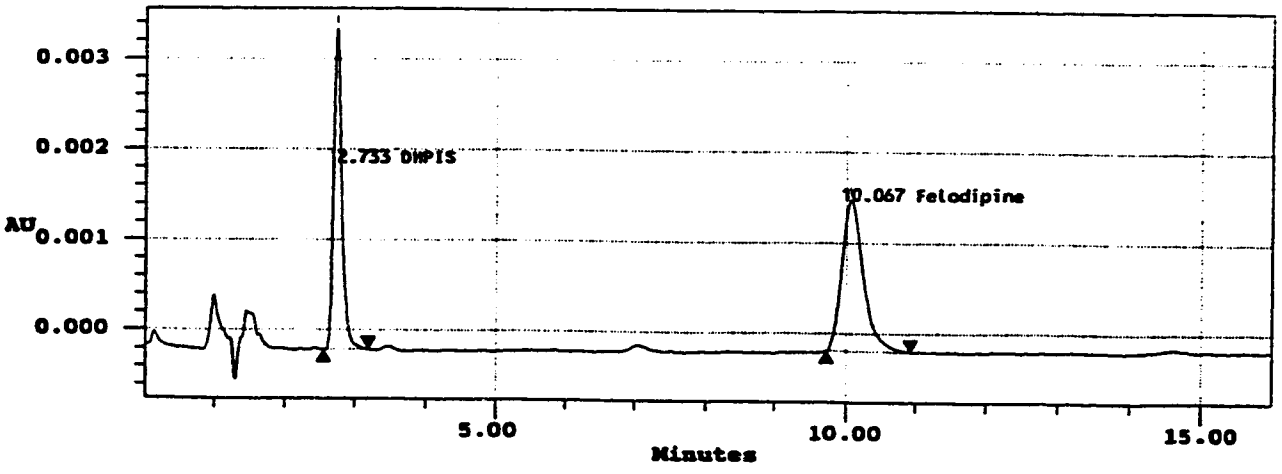


Figure 10. HPLC chromatogram for felodipine. The mobile phase consisted of CH_3CN and water (1:1, v/v) with a flow rate of 1 ml/min. Felodipine had a retention time(R_T) of 10.1 min and the internal standard had a retention time R_T of 2.7 min.

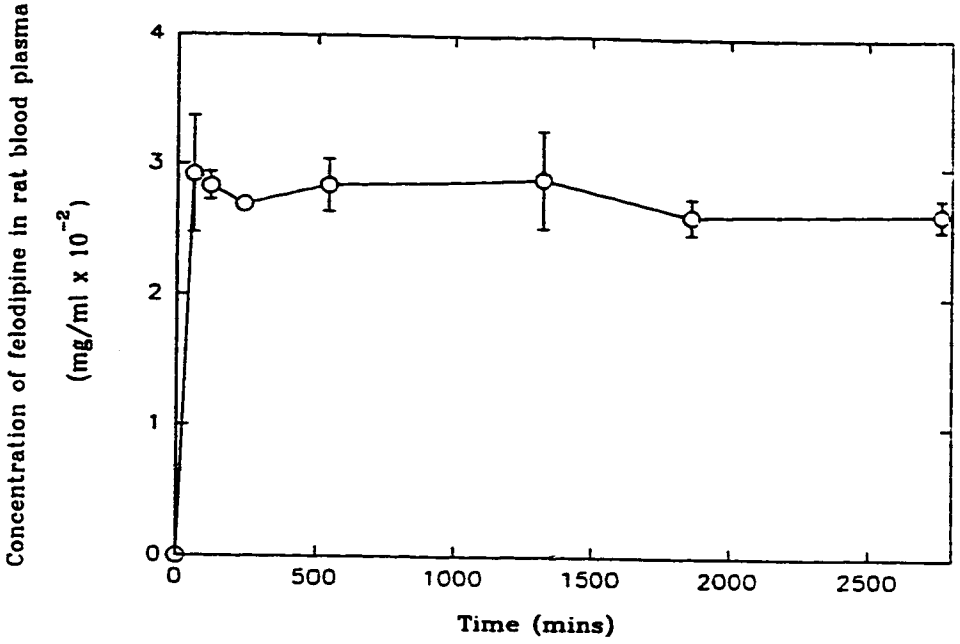


Figure 11a

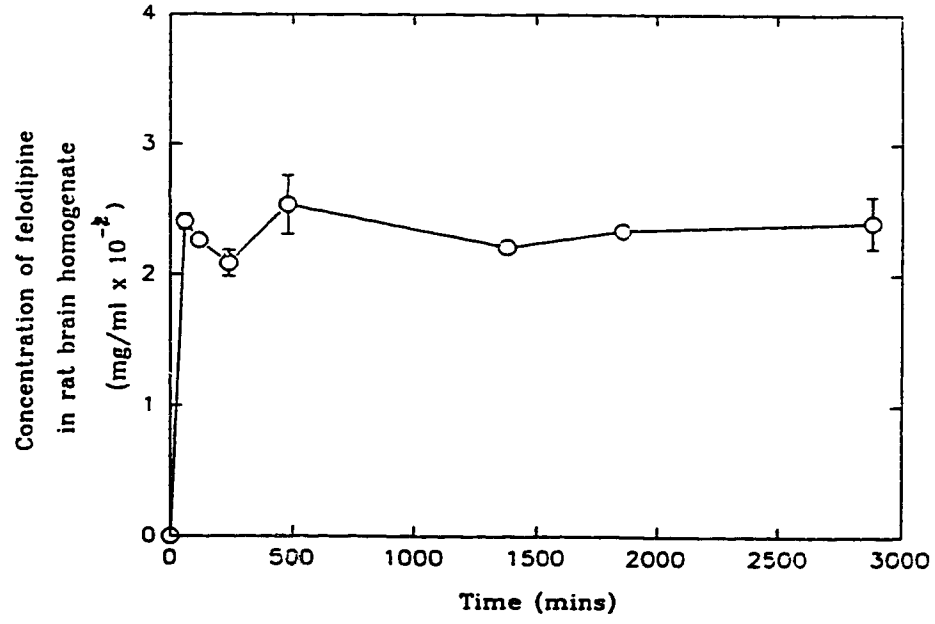


Figure 11b

Figure 11. The stability of felodipine (39) upon incubation with rat plasma at 37°C (a) and 20% rat brain homogenate at 37°C (b). The error bars represent the standard deviation (n = 3)

The results obtained (Figures 11a and 11b) indicate that felodipine (39) is very stable in both plasma and brain homogenate (1:4, w/v in phosphate buffer, pH = 7.4). This is further supported by the fact that felodipine had a 96% recovery in plasma and 85% recovery in brain homogenate. It is likely that felodipine is predominantly metabolized via the liver hepatic microsomal P450 enzyme system.[220]

3.4.1.0.0.0. *In vitro* incubation studies for 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (118) with rat plasma and rat brain homogenate

On the basis of the anticonvulsant test results obtained for the CDS compounds (3.2.6.0.0.0.), the felodipine-CDS (118) was selected for *in vitro* incubation studies. For example, the felodipine-CDS was the only compound in the series which showed activity for both the CDS and its hydroxyethyl derivative (102), although the CDS exhibited a slow onset of action. This study was carried out to determine its stability in rat plasma and in a 20% brain homogenate.

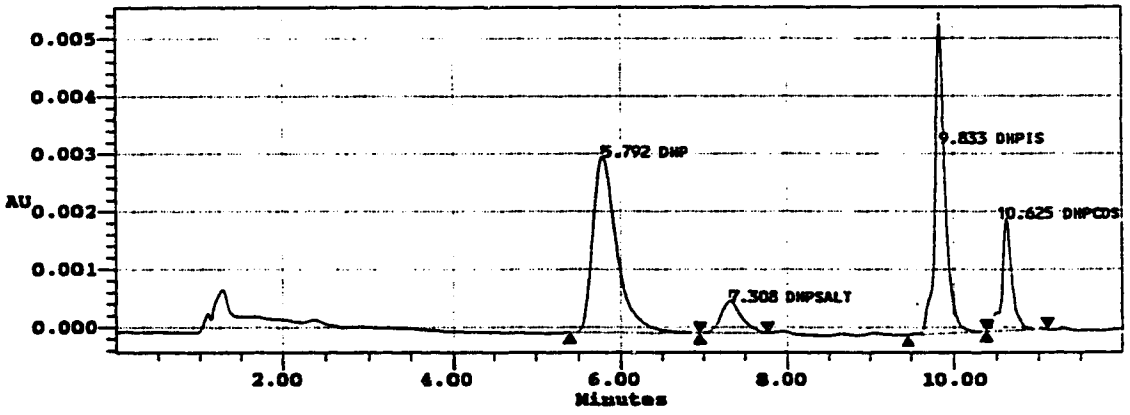


Figure 12. HPLC chromatogram for incubation studies of the felodipine-CDS (118). The mobile phase consisted of CH_3CN-phosphate buffer (0.015 M) using a gradient elution mode starting from 38% CH_3CN to 100% at 20 min. The flow rate started at 1 ml/min until 2 min and it was then changed linearly to 5 ml/min at 20 min. Using a 3.9 x 150 mm reverse phase C_{18} column and UV detection at 350 nm, the retention times for the CDS (118), the quaternary pyridinium salt (115) and the hydroxethyl parent DHP (102) were 10.6 min, 7.3 min and 5.8 min, respectively. The peak at R_T = 9.8 min is the internal standard, 3-(2-methoxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (165).

The concentration (mg/ml) of the CDS (118), the quaternary pyridinium salt (115) and the hydroxyethyl derivative (102) were determined by quantitative HPLC analyses using the conditions described in Figure 12. Since Hantzsch DHPs usually absorb light in the region of 300 - 400 nm[140], a wavelength of 350 nm was used for detection of (118), (115) and (102) in this HPLC assay. The ionic nature of (115) makes detection difficult since it is eluted together with the solvent peak from the reverse phase C_{18} column. Since the retention time (R_T) for (115) was very sensitive to the pH of the mobile phase, an optimum pH of 4.5 for phosphate buffer (NaH_2PO_4, 0.015 M) was determined for the mixed solvent system which eluted (115) as a broad peak at R_T = 7 - 8 min using the gradient separation described earlier (Fig. 12).

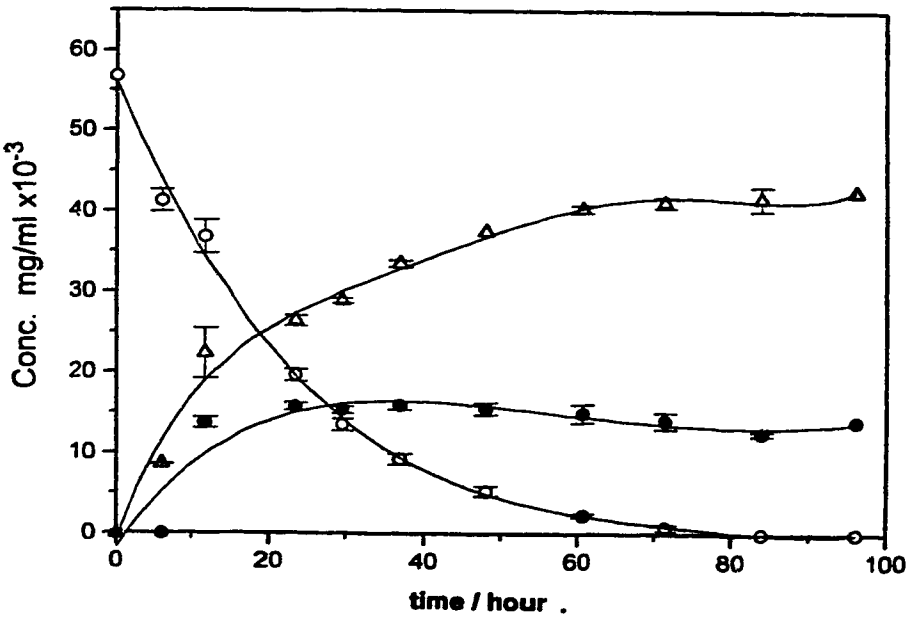


Figure 13(a). The stability of the CDS (118) in rat plasma at 37°C: (o), the CDS (118); (Δ), the hydroxyethyl parent DHP (102) and (●), the quaternary salt (115). The error bars represent the standard deviation (n = 3).

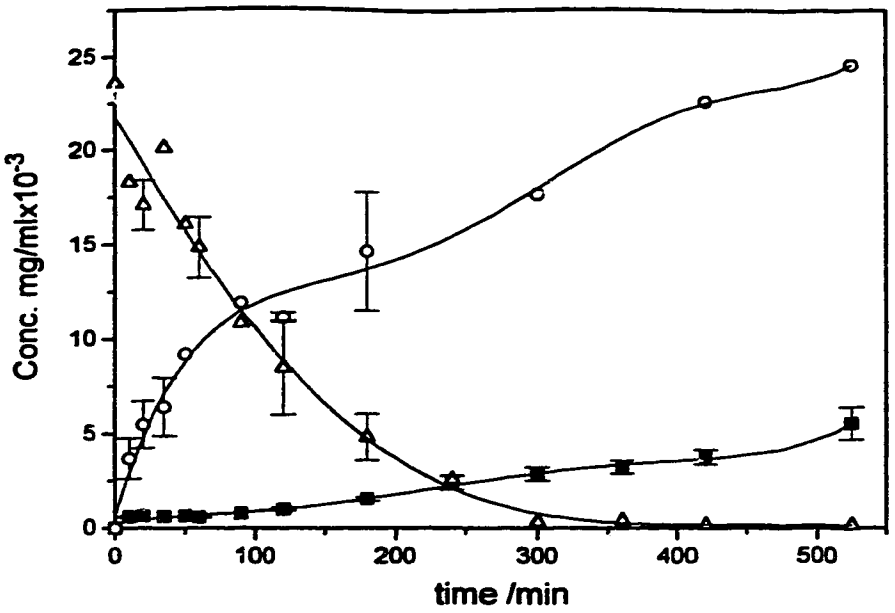


Figure 13(b). The stability of the CDS (**118**) in 20% rat brain homogenate at 37^0 C: (Δ), the CDS (**118**); (■), the hydroxyethyl parent DHP(**102**) and (O), the quaternary salt (**115**). The error bars represent the standard deviation (n = 3).

Table 17. The half-life and rate constant for the disappearance of CDS (**118**) in rat plasma and 20% rat brain homogenate at 37^{0}C

	Half-life (hour)	Rate constant (hour^{-1})
Rat plasma	15.5 (930 min)	0.0447 (7.45x10^{-4} min^{-1})
20% Rat brain homogenate	1.3 (78 min)	0.533 (8.88x10^{-3} min^{-1})

From the graphs shown in Fig. 13a and 13b, the disappearance of the CDS (**118**) was found to be a pseudo-first order reaction in both rat plasma and 20% brain homogenate. The half-lives and the calculated rate constants for the disappearance of (**118**) are listed in Table 17. The results indicate that the CDS (**118**) is more stable in plasma than in the 20% brain homogenate, indicating that systematic administration of the drug should deliver the parent CDS compound to the brain without appreciable oxidation or hydrolysis in the periphery. Although it is not certain whether the

disappearance of CDS (118) is the result of oxidation or hydrolysis, the graphs in Figure 13a suggests a consecutive reaction type kinetics as shown by Equation 27.

$$\text{CDS (118)} \xrightarrow[\text{Oxidation}]{} \text{Pyridinium salt (115)}$$

$$\text{Pyridinium salt (115)} \xrightarrow[\text{Hydrolysis}]{} \text{DHP (102)} \qquad \text{(Equation 27)}$$

These data support the hypothesis that the CDS (118) was first oxidized to the quaternary pyridinium salt (115), which in turn was hydrolysed to the parent hydroxyethyl DHP (102) in both biological media. However, the hydrolysis rate of the quaternary pyridinium salt (115) is much faster in the plasma than in the brain homogenate (Figure 13b).

3.5.0.0.0.0. *In vivo* biodistribution brain uptake studies for felodipine (39)

Felodipine (39) was administered to rats by tail-vein injection. Unlike, the CDS (118), a dose as low as 3.5 mg/kg of felodipine can kill the rat within minutes after injection. This difference in toxicity between the two compounds may be due to the fact that the less neurotoxic CDS (118) was sequestered in the brain as a pyridinium salt, whereas felodipine is either more neurotoxic and/or it exerts peripheral toxicity. Therefore, a 2.5 mg/kg dose of felodipine was employed in this study. At selected times, up to 60 minutes, the rats were sacrificed to determine the concentration (μg/g in rat brain) of felodipine (39) and any possible metabolites. Three rats were used for each time point. The concentraton of felodipine in brain tissue is shown in Figure 14.

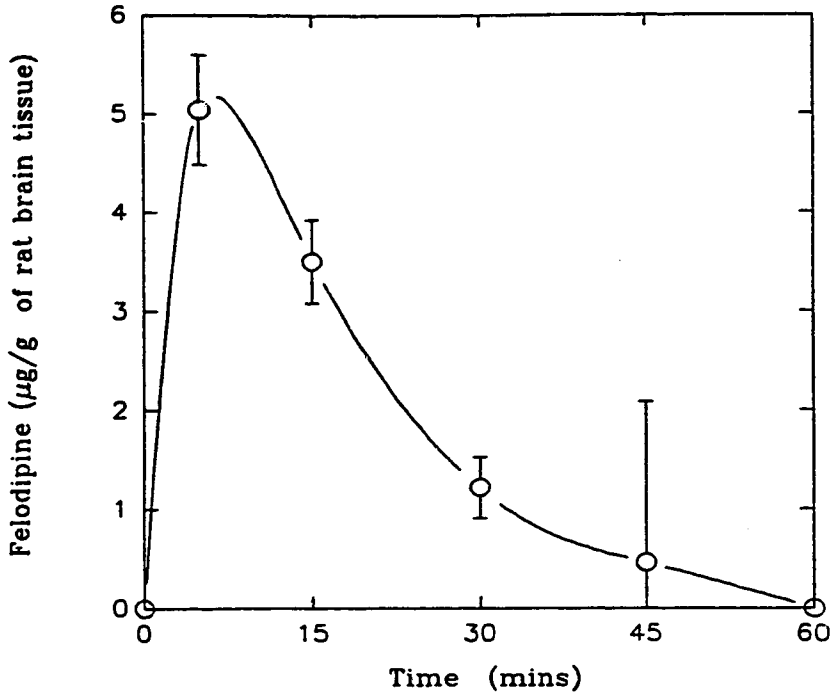


Figure 14. Localization of felodipine (**39**) in whole rat brain after tail-vein injection of felodipine. The error bars represent the standard deviation (n = 3).

The data acquired (Figure 14) show that felodipine enters the brain rapidly and that a maximum brain concentration of 5.03 μg/g brain tissue is achieved at 5 minutes. After 5 minutes, the concentration in brain decreases. At 1 hour postadministration, the drug completely exited the brain. This result is consistent to the finding that felodipine is highly lipophilic (Kp = 442) (Table 2). Enhancement of both the brain entry of felodipine and its subsequent egression from the brain would therefore be expected. With respect to the blood level of felodipine, only one blood sample indicated a trace (0.1 μg/ml) of felodipine, 5 minutes after drug administration. No significant quantity of felodipine could be detected in all other blood samples collected. Hence, no graph depicting felodipine blood concentrations was acquired in this study. Also, HPLC analysis indicated that no metabolites of the 1,4-DHP type were formed for felodipine in brain.

In contrast to this HPLC assay, examination of the anticonvulsant test results for felodipine indicates significant MES protection (2/3) by a 100 mg/kg ip dose at 4 hour postadministration (Table 3), a time which in this assay indicated no detectable drug in both blood and the brain. Whether this discrepancy is due to the difference in conditions in the NIH anticonvulsant screen (a 100 mg/kg ip dose to mice) and this *in vivo* animal study (a 2.5 mg/kg iv dose to rats) is not known. However, a similar discrepancy of anticonvulsant activities for nimodipine with respect to its level in blood and brain of mice was reported.[178]

3.5.1.0.0.0. *In vivo* biodistribution studies of 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (118)

The CDS compound (118) was administered to rats by tail-vein injection. At selected times, up to 96 hours, the rats were sacrificed to determine the concentration of the CDS (118), the quaternary pyridinium salt (115) and the hydroxyethyl compound (102) in both blood and brain by quantitative HPLC analyses. Three rats were used for each time point. A 15 mg/kg dose of the CDS (118) was administered, although occasional toxic side-effects such as ataxia were sometimes observed. The results of this biodistribution study are shown in Figure 15a and 15b.

Although a significant amount of the CDS (118) and the quaternary salt (115) were detected rat blood, both disappeared 2 hours after drug administration (Figure 15a). The hydroxyethyl DHP (102), on the other hand, remained low throughout. These results indicate rapid clearance of the compounds from the periphery.

In the brain, a considerable amount of the CDS (118) was detected (mean value = 4.2 μg/g of brain tissue) at 5 minutes after drug administration (Figure 15b) indicating a rapid uptake of (118). However, it disappeared at a time interval ≥ 15 minutes. Simultaneously, the quaternary salt (115) maintained a high concentration inside the brain even up to 4 days after drug administration. This is in contrast to the hydrolysis product (102) which remained low throughout the time of this study.

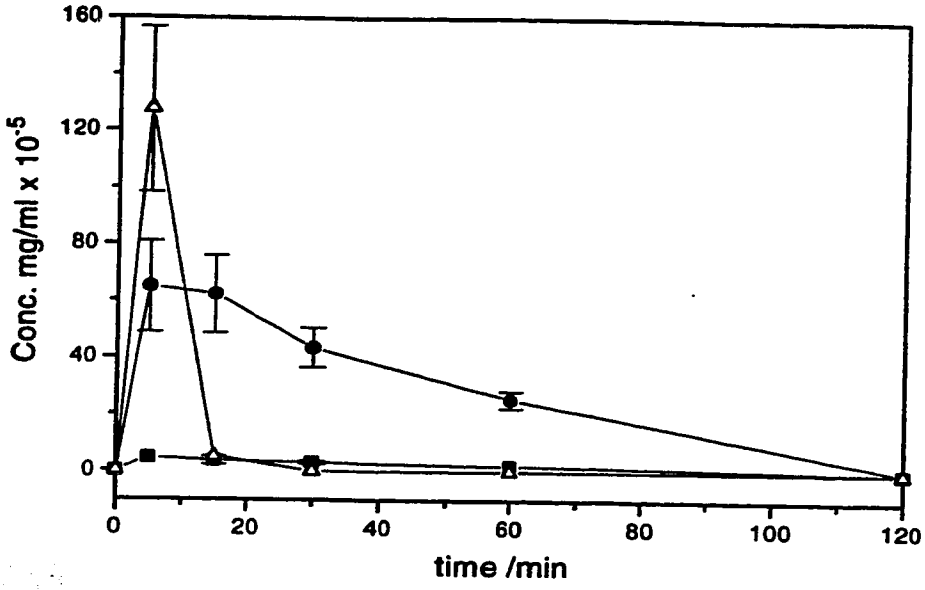


Figure 15a. Concentration of the CDS (118), the quaternary pyridinium salt (115) and the hydroxyethyl DHP (102) in rat blood after tail-vein injection of the CDS (118): (Δ), the CDS (118); (●), the quaternary salt (115) and (■), the hydroxyethyl DHP (102). The error bars represent standard deviation (n = 3).

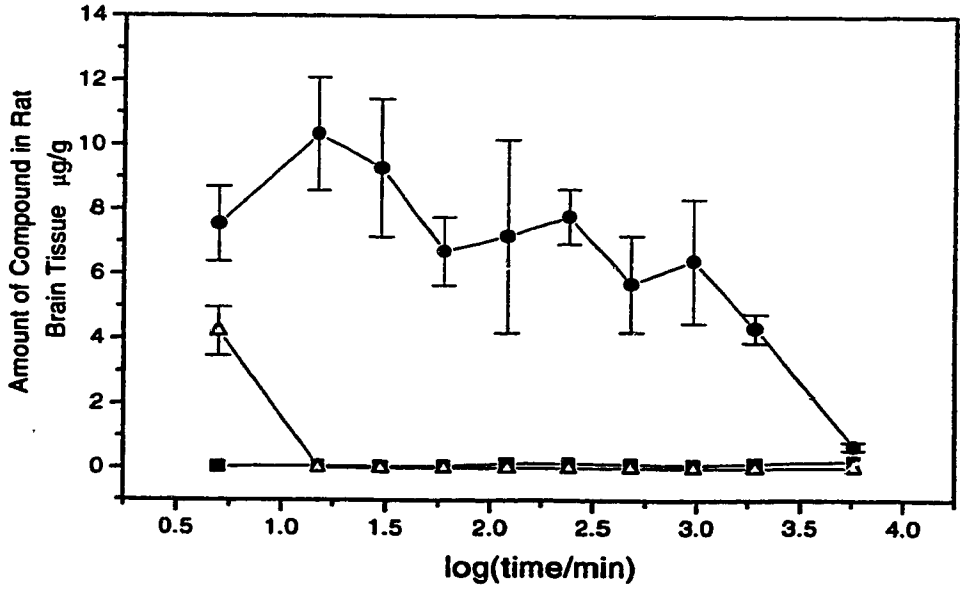
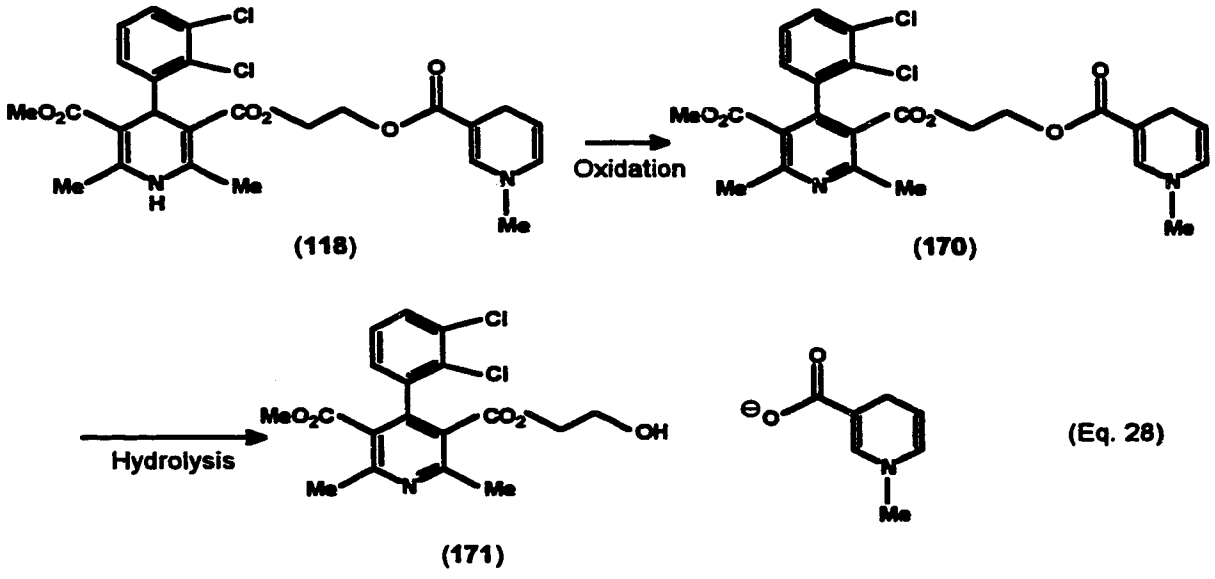


Figure 15b. Concentration of the CDS (118), the quaternary pyridinium salt (115) and the hydroxyethyl DHP (102) in rat brain after tail-vein injection of the CDS (118): (Δ), the CDS (118); (●), the quaternary salt (115) and (■), the hydroxyethyl DHP (102). The error bars represent the standard deviation (n = 3).

In order to offer an explanation for the low concentration of the hydroxyethyl DHP (102) in the brain, several hypotheses can be put forward for consideration. First of all, the low concentration of (102) can be attributed to the lipophilic CDS (118) which may have egressed from the brain before hydrolysis.

Alternatively, the CDS (118) may have entered the brain, but was hydrolyzed to liberate (102) prior to oxidation to trigonelline. The less lipophilic hydroxyethyl DHP (102) would have egressed from the brain and hence a low concentration was recorded.

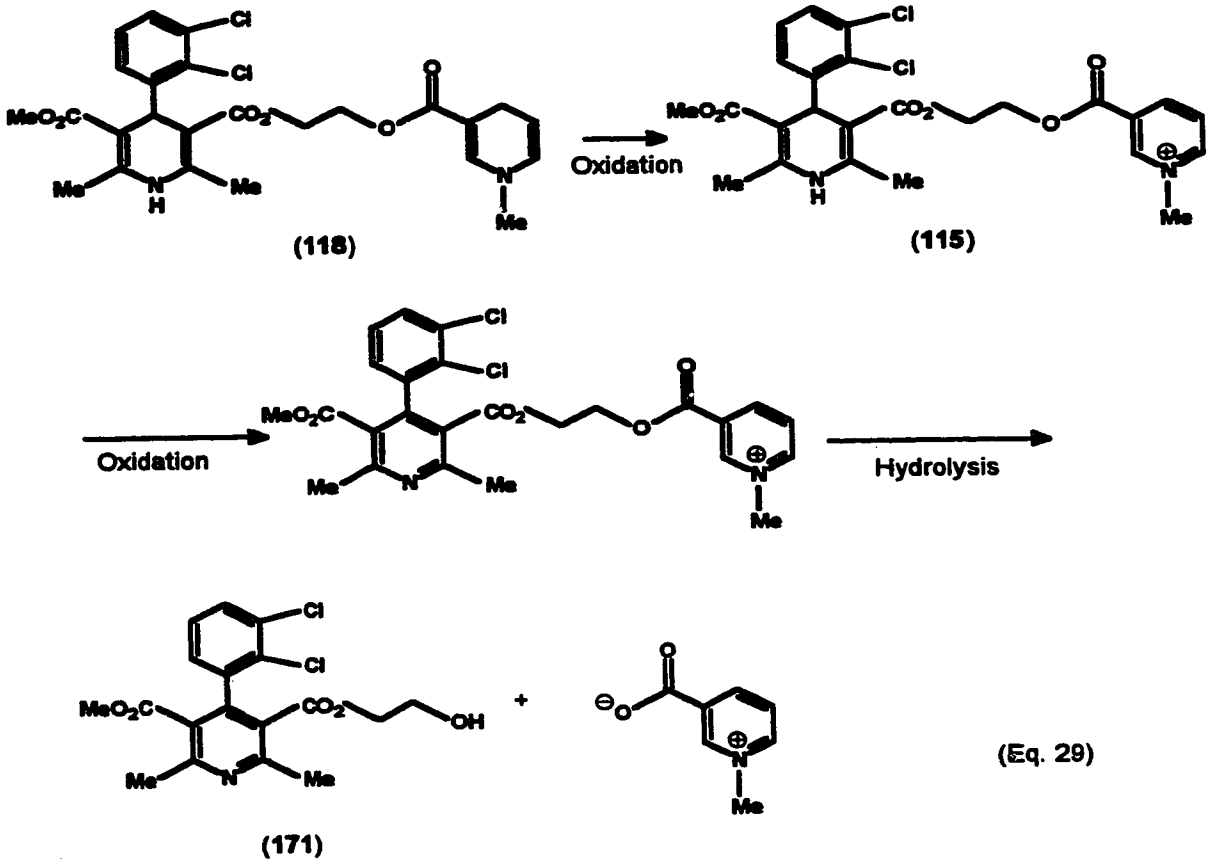
There is also the possibility that the 1,4-dihydropyridine moiety in the parent drug was oxidized prior to the oxidation of the CDS to form a pyridine intermediate (170) which on hydrolysis gives (171) (Equation 28). The low concentration of (102) is therefore due to the product (171) being a pyridine that would not be detected at 350 nm wavelength UV light.



The three hypotheses put forward offer potential explanations for the low concentration of (102). However, a high concentration of the quaternary salt (115) inside the brain is contradictory to the assumptions of the hypotheses, suggesting that they are unlikely to be true.

It may be argued that the CDS (118) was first converted to the quaternary salt (115) and was then followed by the oxidation of the 1,4-dihydropyridine moiety of the

parent drug as indicated by Equation 29. Again, the hydrolysed product (171) was not detected because of its pyridine moiety.



(118) (115) (171) (Eq. 29)

This hypothesis offers a reasonable explanation for the low concentration of (102) and at the same time is not contradictory to the graphical results in Figure 15b. However, the fact that felodipine is very stable to oxidation in the *in vitro* studies (3.4.0.0.0.0.) is not consistent with the assumptions offered since (102) is an analog of felodipine.

Therefore, we assume that in this study, the CDS(118) was rapidly delivered to the brain where it was oxidized to the quaternary salt (115). The polar nature of the salt (115) resulted in its being locked-in the brain. The low concentration of (102) may be a result of slow hydrolysis and/or the egression of it from the brain which can happen as soon as it is formed. This is consistent with the *in vitro* study for (118) with brain homogenate (3.4.1.0.0.0.) which indicated a slower rate of hydrolysis. While the oxidation of the 1,4-dihydropyridine moiety of (102) cannot be ruled out, the fact that similar oxidation of the

1,4-dihydropyridine in the quaternary salt (115) did not occur (if it did, then the concentration of the salt would decline rapidly) may suggest that this may not be a likely factor for consideration.

Although the nature of the metabolism of CDS (118) is not clearly determined, the low concentration of (102) may offer an explanation for the slow onset of action of the (118) in the anticonvulsant screen as time is required for an effective concentration of the hydroxyethyl DHP (102) to be formed to provide seizure protection. This study may also explain the inactivity/low anticonvulsant activity of other Bodor CDS compounds investigated as anticonvulsant agents which may also undergo a slow hydrolysis of the pyridinium salt.

In order to increase the hydrolysis rate of pyridinium salt, which is likely to be the factor for low concentration of (102), a double ester prodrug[199] with a single carbon-spacer between the two ester moieties similar to the valproate DHP (98) described in earlier studies (3.1.3.0.0.0.) could be considered. Although (98) was toxic (3.2.4.0.0.0.), the same may not be true for the 1-methyl-1,4-dihydropyridyl-3-carbonyloxy CDS system. In fact, a double ester prodrug of this type was successful in elevating the concentration of a β-lactam[223] in blood and other tissues due to a fast hydrolysis for this particular pro-drug. In addition, replacing the 1-methyl substituent on the 1,4-dihydropyridyl CDS by an alkyl or an aryl group could be investigated. For example, a structure-activity relationship study[224] for a series of reduced nicotinamide derivatives as anticonvulsant agents show that the 1-octyl or 1-ethyl-1,4-dihydronicotinamides were most potent in attenuating the oxidation of brain mitochondrial NADH which was considered as a mechanism for seizure spread. The 1-methyl-1,4-dihydropyridyl moiety used in this study is the first report describing its application in conjuction with a calcium channel antagonist for seizure protection.

3.6.0.0.0.0. Conclusions

The results acquired in this study show that some brain-targeted DHPs offer some protection against seizures. These results also reinforce the concept that calcium is involved in seizure generation (1.4.0.0.0.0.), although this is not likely the sole factor for

its pathogenesis. The fact that the DHPs described in this study are not very effective in the scMet screen may indicate that the role of calcium, other than at L-type calcium channels, may be involved in absence seizures.[51,52,60]

The 1-methyl-1,4-dihydropyridyl-3-carbonyloxy CDS is a viable method to enhance the entry of DHPs into brain and that rapid oxidation of this moiety prolongs the duration of action of the drug provided that the ester linkage of the pyridinium salt undergoes rapid hydrolysis. The slow onset of action of the felodipine-CDS, with respect to anticonvulsant activity, is likely due to the slow hydrolysis of the pyridinium salt. Further drug design studies in this area must address measures to increase the ester hydrolysis rate for the pyridinium salt species.

3.6.1.0.0.0. Structure-activity relationships for 3-(2-*n*-propylpentanoyloxyalkyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (98 -101)

1. CCA activity is generally decreased by the addition of a valproate moiety to the C-3 hydroxyalkyl substituent.
2. The Kp value is increased upon elaboration to a C-3 valproate ester.
3. The chain length (n) of the alkyl chain in the C-3 ester is a determinant of CCA activity where the activity profile is n = 1 > 3 > 2.
4. A single-carbon spacer between the two ester moieties in the C-3 substituent of (98) increases toxicity which would preclude its use as an anticonvulsant agent.
5. The two-carbon spacer ester prodrugs (99) and (100) are both active in the MES screen. Compound (99), which has a C-5 methyl ester substituent, is devoid of toxic side-effects, whereas compound (100) which has a C-5 isopropyl ester moiety is highly toxic at a 100 mg/kg ip dose.
6. The three-carbon spacer ester prodrug (101) was an inactive anticonvulsant agent.

3.6.2.0.0.0. Structure-activity relationships for 3-pentanoyloxyethyl 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (108 - 109)

1. A C-3 valerate ester moiety markedly increases the CCA activity of these DHPs.
2. The CCA activity profile for these valerate prodrugs with respect to the C-5 alkyl ester is methyl > isopropyl.
3. The valerate ester prodrugs (108 - 109) were moderately active as anticonvulsant agents.

3.6.3.0.0.0. Structure-activity relationships for 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (118 - 120) and 3-ethyl 5-methyl 1,4-dihydro-2-[2-[(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethoxy]methyl]-6-methyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (131)

1. Introduction of the 1-methyl-1,4-dihydropyridyl-3-carbonyloxyethyl CDS to a DHP has a minimal effect on CCA activity.
2. The CCA activity potency order for substituents on the C-4 phenyl ring was 3-Br > 2,3-Cl_2 > 3-CF_3 in these CDS prodrugs, whereas the order for the hydroxyethyl DHP is 2,3-Cl_2 > 3-CF_3 > 3-Br.
3. CDS compounds (120) and (131) are inactive anticonvulsant agents as compared to their parent DHPs (111) and (128). This observation may be due to slow hydrolysis of the CDS ester pyridinium salt.
4. There is an improved anticonvulsant activity for the 3-trifluoromethylphenyl CDS compound (119) (class 2) compared to its parent DHP (110) (class 3).
5. Anticonvulsant activity for the hydroxyethyl DHP series of compounds with respect to the C-4 phenyl substituent was 2,3-Cl_2 (class 1) > 3-Br (class 2) > 3-CF_3 (class 3).
6. The amlodipine analog (128) having a 2-hydroxyethoxymethyl substituent at the C-2 position is an active anticonvulsant agent with a longer duration of action (4 hours).

3.6.4.0.0.0. Anticonvulsant evaluation for 3-[2-[4-(4-fluorophenyl)piperazinyl]ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (137)

1. The CCA activity of (137) (IC_{50} = 1.59 x 10^{-8} M) is comparable to that of nimodipine.
2. This type of prodrug is too toxic to be used as an anticonvulsant agent.

3.6.5.0.0.0. Structure-activity relationships for 3-[(2-dimethylamino)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (139) and 3-[2-(trimethylammonium)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate iodide (140)

1. A quaternary ammonium moiety reduces CCA activity by 3 orders of magnitude.
2 Quaternization of the amine moiety markedly reduces the Kp value of the DHP compound (140).
3. Both types of prodrugs (139) and (140) are too toxic to be used as anticonvulsant agents. Their toxicity may be due to cholinergic agonist actions.

3.6.6.0.0.0. Structure-activity relationships for 3-[4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (144) and 3-[4,4-*bis*-(*o*-tolyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (148)

1. The *bis*-(*o*-tolyl)-3-butenyl prodrug (148) is more potent than the *bis*-(3-methyl-2-thienyl)-3-butenyl prodrug (144) with respect to CCA activity.
2. The *bis*-(3-methyl-2-thienyl)-3-butenyl prodrug (144) is more lipophilic than the *bis*-(*o*-tolyl)-3-butenyl prodrug (148).
3. Both types of prodrugs (144) and (148) are inactive anticonvulsant agents.

3.6.7.0.0.0. Structure-activity relationships for 3-(2-methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (150, 152, 154) and 3,5-diisopropyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (151, 153, 155)

1. A 3- or 4-dimethylaminophenyl substituent lowers the lipophilicity (Kp = 104 - 137 range) relative to a 3,4,5-trimethoxyphenyl substitutent (Kp = 208 - 259 range).
2. The CCA activity profile for C-4 substituted phenyl analog is *meta*-NMe_2 > *para*-NMe_2 > 3,4,5-trimethoxy.
3. The symmetrical diisopropyl ester DHPs are generally more potent CCAs than the corresponding racemic 3-(2-methoxyethyl) analogs, except for the 3,4,5-trimethoxyphenyl compounds (154, 155).
4. Compounds in this class of agents exhibited marginal anticonvulsant activity where the activity order with respect to C-4 phenyl substituents was generally *meta*-NMe_2 > *para*-NMe_2 > 3,4,5-trimethoxy.

4.0.0.0.0. EXPERIMENTAL SECTION

4.1.0.0.0. Physical constants and spectroscopy

Melting points were determined in capillary tubes using a Thomas Hoover melting point apparatus and are uncorrected. IR spectra were recorded using a Nicolet 5DX - FT spectrometer. Solid samples were prepared as KBr pellets and liquid samples were used neat between NaCl plates. Nuclear magnetic resonance spectra (^{1}H NMR) were acquired using a Bruker AM - 300 spectrometer using either $CDCl_3$ or DMSO-d_6 as solvent. Quantitative UV analyses, to determine partition coefficients, were performed using either a PU 8700 Series UV/visible spectrophotometer or LKB Ultrospec 4050 UV spectrophotometer. Microanalyses were performed by the Microanalytical Laboratory, Department of Chemistry, University of Alberta.

4.2.0.0.0. Chromatography

Thin Layer Chromatography (TLC), to monitor reaction progress, was conducted using 0.25 mm POLYGRAM SIL G/UV_{254} silica gel plates with UV light visualization (254 nm). Column chromatography, for purification of products, was performed using Merck silica gel 60 ASTM (70 - 230 mesh) with either ethyl acetate-hexane or dichloromethane-methanol as eluent.

All High Performance Liquid Chromatography (HPLC) analyses were performed using a Waters HPLC system comprised of two Model 510 pumps, Model U6K injector, Model 486 tunable absorbance detector, Millennium 2010 software and a Waters analytical dimethyloctadecylsilyl Nova Pak® C18 reverse phase column (3.9 x 150 mm). UV detection at 350 nm was used for all the analytical studies described. Acetonitrile and water (HPLC grade) / phosphate buffer (0.015 M) were used as mobile phase which were filtered through a 0.45 μm filter (Millipore® from Waters) prior to the analyses. Degassing by sparging helium gas into both solvents were employed during the analyses.

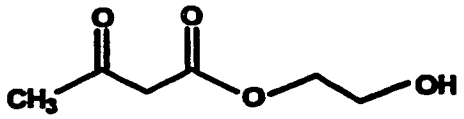
4.3.0.0.0.0. Solvents and reagents

Tetrahydrofuran (THF) and diethyl ether were dried by refluxing in the presence of sodium and benzophenone before distillation. Ethylene glycol was dried by stirring with magnesium sulphate prior to distillation *in vacuo*. Dichloromethane was dried by distillation from calcium hydride and absolute ethanol was obtained by refluxing with magnesium prior to distillation. Organometallic reagents were purchased in "sure-sealed" containers from the Aldrich Chemical Co.

4.4.0.0.0.0. Synthetic chemistry

4.4.1.0.0.0. Synthesis of 2-hydroxyethyl acetoacetate (161)

General procedure for the synthesis of alkyl acetoacetates (Procedure A)



(161)

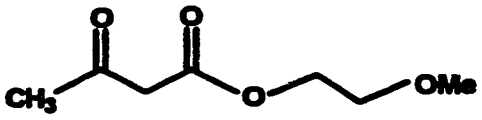
Freshly distilled diketene (8.15 g, 97 mmol) was added dropwise to a solution of dry ethylene glycol (24.05 g, 387 mmol) and triethylamine (0.25 ml, 4.6 mmol) at 60°C with stirring. Since this reaction is exothermic, diketene was added at a rate such that the temperature of the reaction mixture did not exceed 80°C. After the addition was completed, the reaction was allowed to proceed at 95°C for an additional 3 hours. The product was isolated by silica gel column chromatography using ethyl acetate-hexane (2:1, v/v) as eluent. The first product eluted was the *bis*-substituted glycol (0.65 g, 2.8 mmol). Further elution afforded the product (**161**) as a light yellow liquid (11.9 g, 84%).

^{1}H NMR ($CDCl_3$): δ 4.24 (t, J = 4.6 Hz, 2H, $COOCH_2$), 3.78 (t, J = 4.6 Hz, 2H, CH_2OH), 3.49 (s, 2H, $COCH_2CO$), 2.92 (br, s, 1H, OH, exchanges with D_2O), 2.24 (s, 3H, CH_3CO).

IR (film): 3074 - 3706 (br) (OH), 1760 (C=O, ester), 1720 (C=O, ketone) cm^{-1}.

The product (**161**) was used immediately for the synthesis of several DHPs to be described later.

4.4.1.1.0.0. 2-Methoxyethyl acetoacetate (73)



(73)

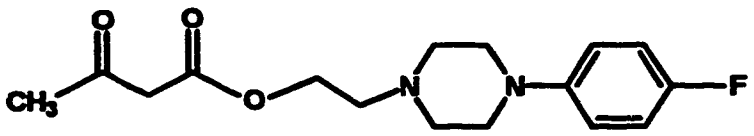
Compound (73) was prepared according to Procedure A by reaction of 2-methoxyethanol (2.73 g, 35.9 mmol), diketene (3.02 g, 35.9 mmol) and triethylamine (0.5 ml, 9.2 mmol). The product (73) was isolated by distillation [bp 82 - 84°C (3 mm Hg)] Lit.[194] [bp 110 - 116°C (14 mm Hg)] as a colourless liquid (5.06 g, 88%).

^{1}H NMR ($CDCl_3$): δ 4.26 (t, J = 4.7 Hz, 2H, $COOCH_2$), 3.57 (t, J = 4.7 Hz, 2H, CH_2OMe), 3.46 (s, 2H, $COCH_2CO$), 3.34 (s, 3H, OCH_3), 2.23 (s, 3H, CH_3CO).

IR (film): 1752 (C=O, ester), 1727 (C=O, ketone) cm^{-1}.

This intermediate compound was used immediately for the synthesis of nimodipine (38) and several DHPs to be described later.

4.4.1.2.0.0. 2-[4-(*p*-Fluorophenyl)piperazin-1-yl]ethyl acetoacetate (136)



(136)

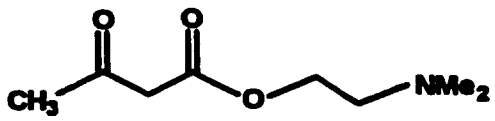
The method used was similar to that described in Procedure A. Reaction of 2-[4-(*p*-fluorophenyl)]piperazin-1-yl] ethanol (135) (2.42 g, 10 mmol) and diketene (0.84 g, 10 mmol) and triethylamine (0.5 ml, 9.2 mmol) gave a product which was isolated by silica gel column chromatography with CH_2Cl_2-MeOH (96:4, v/v) as eluent. The product (136) was isolated as a yellow oil (2.62 g, 85%).

^{1}H NMR ($CDCl_3$) δ 6.82 - 6.97 (m, 4H, aryl-H), 4.30 (t, J = 5.8 Hz, 2H, $COOCH_2$), 3.47 (s, 2H, $COCH_2COO$), 3.09 (t, J = 4.9 Hz, 4H, piperazinyl H-3, H-5), 2.63 - 2.73 (m, 6H, $COOCH_2CH_2$ and piperazinyl H-2, H-6), 2.27 (s, 3H, CH_3CO).

IR (film): 1776 (C=O, ester), 1720 (C=O, ketone), 1229 (C-F) cm^{-1}.

Compound (136) was used immediately for the synthesis of (137).

4.4.1.3.0.0. 2-(N,N-Dimethylamino)ethyl acetoacetate (138)



(138)

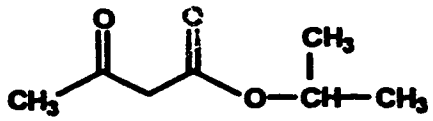
The title compound (138) was prepared according to Procedure A by using N,N-dimethylethanolamine (8.91 g, 100 mmol), diketene (8.41 g, 100 mmol) and triethylamine (0.5 ml, 9.2 mmol). The reaction mixture was purified by distillation *in vacuo* [bp 98 - 99°C (3mm Hg)] to yield (138) as a colourless liquid (14.6 g, 84.3%).

^{1}H NMR ($CDCl_3$): δ 4.20 (t, J = 5.7 Hz, 2H, $COOCH_2$), 3.45 (s, 2H, $COCH_2COO$), 2.53 (t, J = 5.7 Hz, 2H, CH_2NMe_2), 2.23 (s, 9H, CH_3CO and NCH_3).

IR (film): 1745 (C=O, ester), 1726 (C=O, ketone) cm^{-1}.

This compound was used for the synthesis of (139) and (140).

4.4.1.4.0.0. Isopropyl acetoacetate (161)



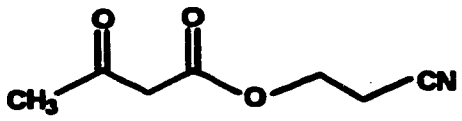
(161)

The product (161) was prepared by the method described in Procedure A by reaction of isopropanol (6.99 g, 116.3 mmol), diketene (9.78 g, 116.3 mmol) and triethylamine (0.5 ml, 9.2 mmol). The product (161) was isolated by distillation *in vacuo* [bp 39 - 41°C (3 mm Hg)] Lit.[194] [bp 66 - 69°C (10 mm Hg)] as a colourless liquid (15.1 g, 90%).

^{1}H NMR ($CDCl_3$): δ 5.06 (septet, J = 6.1 Hz, 1H, $CHMe_2$), 3.41 (s, 2H, $COCH_2COO$), 2.26 (s, 3H, CH_3CO), 1.26 (d, J = 6.1 Hz, 6H, $CHCH_3$).

IR (film): 1742 (C=O, ester), 1727 (C=O, ketone) cm^{-1}.

4.4.1.5.0.0. 2-Cyanoethyl acetoacetate (162)



(162)

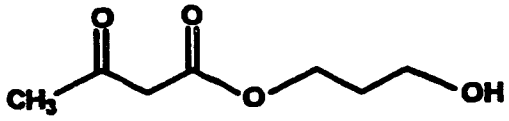
This synthesis was carried out according to the method described in Procedure A by reaction of 3-hydroxypropionitrile (7.11 g, 100 mmol) and diketene (8.41 g, 100 mmol) in the presence of triethylamine (0.5 ml, 9.2 mmol). Since this reaction is highly exothermic, diketene was added at 25°C to 3-hydroxypropionitrile at such a rate that the temperature did not rise above 60°C. The reaction mixture was then heated at 90°C for 3 hours and the product was purified by silica gel column with ethyl acetate-hexane (1:1 v/v) as eluent to afford (162) as a light yellow liquid (11.4 g, 73%).

^{1}H NMR ($CDCl_3$): δ 4.31 (t, J = 6.2 Hz, 2H, $COOCH_2$), 3.50 (s, 2H, $COOCH_2CO$), 2.71 (t, J = 6.2 Hz, 2H, CH_2CN), 2.25 (s, 3H, CH_3CO).

IR (film): 2254 (CN), 1757 (C=O, ester), 1720 (C=O, ketone) cm^{-1}.

The product (162) was used immediately for the synthesis of selected DHPs to be described later.

4.4.1.6.0.0. 3-Hydroxypropyl acetoacetate (163)



(163)

The title compound (163) was prepared according to Procedure A by reaction of propane-1,3-diol (15.22 g, 200 mmol) and diketene (4.2 g, 50 mmol) in the presence of triethylamine (0.25 ml, 4.6 mmol). The product was isolated by elution from a silica gel column with ethyl acetate-hexane (2:1, v/v) as eluent to give (163) as a light yellow liquid (7.44 g, 93%).

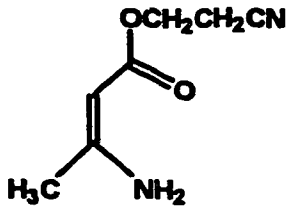
^{1}H NMR ($CDCl_3$): δ 4.29 (t, J = 6.1 Hz, 2H, $COOCH_2$), 3.70 (t, J = 6.1 Hz, 2H, CH_2OH), 3.47 (s, 2H, $COOCH_2CO$), 2.26 (s, 3H, CH_3CO), 2.36 (s, 1H, OH), 1.88 (pentet, J = 6.1 Hz, 2H, $CH_2CH_2CH_2$).

IR (film): 3701 - 3057 (br) (OH), 1744 (C=O, ester), 1720 (C=O, ketone) cm^{-1}.

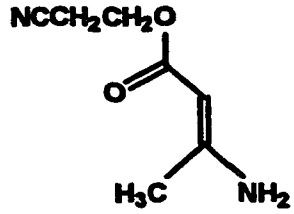
The product (**163**) was used immediately for the synthesis of (**104**).

4.4.2.0.0.0. Synthesis of 2-cyanoethyl 3-aminocrotonate (*E/Z* mixture, ratio 1:4.7) (89)

General procedure for the synthesis of alkyl 3-aminocrotonates (Procedure B)



(*Z*)-(**89**)



(*E*)-(**89**)

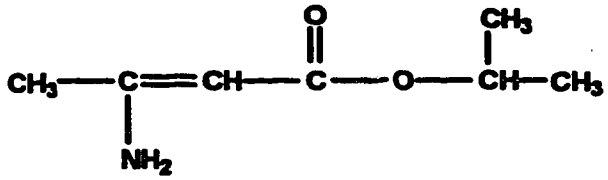
Freshly distilled diketene (5.04 g, 0.06 mol) was added to a solution of 3-hydroxypropionitrile (4.26 g, 0.06 mol) and triethylamine (0.25 ml, 4.6 mmol) at 25°C with stirring. Since the reaction was highly exothermic, the rate of addition must be very slow so that the temperature of the reaction mixture did not exceed 70°C. After the addition of diketene was completed, the reaction mixture was heated at 85 - 90°C for 3 hours. The product, 2-cyanoethyl acetoacetate was not isolated. Dry ammonia gas was then bubbled into the solution for one hour at 25°C with stirring. The yellow precipitate which formed was then filtered off and washed with ether-hexane (1:1, v/v) (3 x 10 ml). Recrystallization from CH_2Cl_2-hexane yielded a mixture of (*E* and *Z*) (**89**) as colourless crystals (6.63 g, 68.7%); mp 92 -94°C. The ratio of (*E*)-(**89**) and (*Z*)-(**89**), as determined from the integrals of the methyl resonances at δ 2.29 and δ 1.94 was 1 : 4.7. The assignment of (*E*)- and (*Z*)-isomers is based on the ^{1}H NMR spectra. The methyl protons of the (*E*)-isomer were deshielded by the carbonyl group of the *cis*-oriented ester moiety and hence resonate at lower field (δ 2.29) whereas the methyl protons of the (*Z*)-isomer were not affected and so resonate at higher field (δ 1.94).

^{1}H NMR ($CDCl_3$) for (*Z*)-isomer: δ 7.4 - 8.2 (br s, 2H, N*H*$_2$, exchanges with D_2O), 4.56 (s, 1H, vinyl-H), 4.27 (t, J = 6.4 Hz, 2H, COOC*H*$_2$), 2.70 (t, J = 6.4 Hz, 2H, C*H*$_2$CN), 1.94 (s, 3H, C*H*$_3$).

^{1}H NMR ($CDCl_3$) for (*E*)-isomer: δ 4.4 - 4.9 (br s, 2H, N*H*$_2$, exchanges with D_2O), 4.36 (t, J = 6.4 Hz, 2H, COOC*H*$_2$), 3.54 (s, 1H, vinyl-H), 2.75 (t, J = 6.4 Hz, 2H, C*H*$_2$CN), 2.29 (s, 3H, C*H*$_3$CO).

IR (KBr): 3324 (NH), 2256 (CN), 1657 (C=O) cm^{-1}.

4.4.2.1.0.0. Isopropyl 3-aminocrotonate (74)



(74)

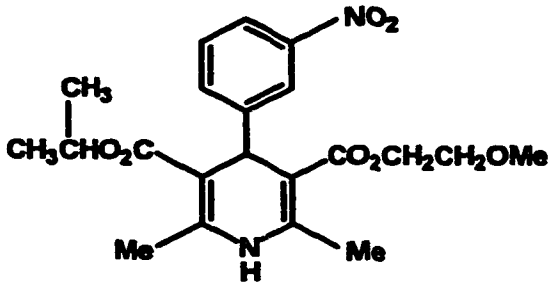
The title compound (74) was prepared according to the method described in Procedure B by bubbling ammonia into neat isopropyl acetoacetate (161) (6.25 g, 43.4 mmol). The product was isolated by distillation *in vacuo* [bp 67 - 70^{0}C (3 mm Hg)] Lit[194] [bp 102 - 103°C (12 mm Hg)] as a colourless liquid which solidified at low temperature (4 - 5°C) (5.4 g, 87 %).

^{1}H NMR ($CDCl_3$): δ 7.0 - 8.4 (br, N*H*$_2$, exchanges with D_2O), 4.98 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.47 (s, 1H, vinyl-H), 1.87 (s, 3H, C-3 C*H*$_3$), 1.21 (d, J = 6.2 Hz, 6H, CHC*H*$_3$).

IR (film): 3441, 3334 (NH), 1637 (C=O) cm^{-1}.

4.4.3.0.0.0. Synthesis of 3-(2-methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (nimodipine) (38)

General procedure for the synthesis of Hantzsch dihydropyridines by a three component reaction (Procedure C)



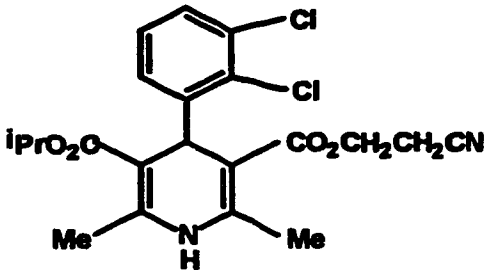
(38)

A solution of 3-nitrobenzaldehyde (**72**) (2.89 g, 19.1 mmol), isopropyl 3-aminocrotonate (**74**) (2.74 g, 19.1 mmol) and 2-methoxyethyl acetoacetate (**73**) (3.06 g 19.1 mmol) in EtOH (95% 80 ml) was refluxed for 16 hours. After removal of the solvent *in vacuo*, the residue obtained was purified by SiO_2 column chromatography using ethyl acetate-hexane (1:3, v/v) as eluent. The product obtained was recrystallized from ethyl acetate-hexane to afford (**38**) as yellow crystals. (5.5 g 69%); mp 124 - 125.5°C Lit[194] mp 125 - 126°C.

^{1}H NMR ($CDCl_3$): δ 8.14 (t, J = 1.2 Hz, 1H, aryl H-2), 8.01 (ddd, J = 9.0 Hz, J = 1.2 Hz, J = 1.2 Hz, 1H, aryl H-4), 7.67 (ddd, J = 9.0 Hz, J = 1.2 Hz, J = 1.2 Hz, 1H, aryl H-6), 7.38 (t, J = 9.0 Hz, 1H, aryl H-5), 5.68 (br s, 1H, NH), 5.10 (s, 1H, H-4), 4.95 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.17 (m, 2H, COOC*H*$_2$), 3.55 (m, 2H, C*H*$_2$OMe), 3.36 (s, 3H, OC*H*$_3$), 2.37 (s, 6H, C-2 and C-6 C*H*$_3$), 1.26 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.09 (d, J = 6.2 Hz, 3H, CHC*H*$_3$)

IR (KBr): 3312 (NH), 1696 (C=O), 1532, 1352 (NO_2) cm^{-1}.

4.4.3.1.0.0. 3-(2-Cyanoethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (96)



(96)

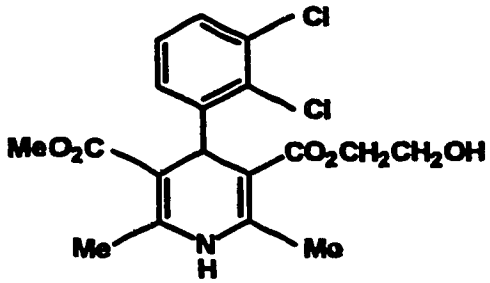
Compound (**96**) was prepared according to the method described in Procedure C by reaction of 2,3-dichlorobenzaldehyde (7.0 g, 40 mmol), isopropyl 3-aminocrotonate (**74**) (5.73 g, 40 mmol) and 2-cyanoethyl acetoacetate (**162**) (6.2 g, 40 mmol). The product was purified by elution from a silica gel column using ethyl acetate-hexane (3:7, v/v)as eluent to afford the product (**96**) as a yellow oil (9.9 g, 57%).

^{1}H NMR ($CDCl_3$): δ 7.33 (dd, J = 7.5 Hz, J = 1.2 Hz, 1H, aryl H-4), 7.27 (dd, J = 7.5 Hz, J = 1.2 Hz, 1H, aryl H-6), 7.09 (t, J = 7.5 Hz, 1H, aryl H-5), 5.84 (br s, 1H, N*H*), 5.43 (s, 1H, H-4), 4.97 (septet, J = 6.3 Hz, 1H, C*H*Me$_2$), 4.24 (t, J = 6.5 Hz, 2H, COOC*H*$_2$), 2.65 (t, J = 6.5 Hz, 2H, C*H*$_2$CN), 2.32 and 2.31 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.26 (d, J = 6.3 Hz, 3H, CHC*H*$_3$), 1.05 (d, J = 6.3 Hz, 3H, CHC*H*$_3$).

IR (film): 3346 (NH), 2255 (CN), 1666 (C=O) cm^{-1}.

The product (**96**) was used immediately for the synthesis of (**94**).

4.4.3.2.0.0. 3-(2-Hydroxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (102)



(102)

The title compound (**102**) was prepared according to Procedure C by reaction of 2,3-dichlorobenzaldehyde (1.80 g, 10.3 mmol), 2-hydroxyethyl acetoacetate (**160**) (1.50 g, 10.3 mmol) and methyl 3-aminocrotonate (1.18 g, 10.3 mmol). The product was eluted from a silica gel column using ethyl acetate-hexane as eluent (1:1, v/v) to afford (**102**) as a solid which was recrystallized from CH_2Cl_2-hexane as a light yellow crystalline solid (2.0 g, 48%); mp 118-132^{0}C.

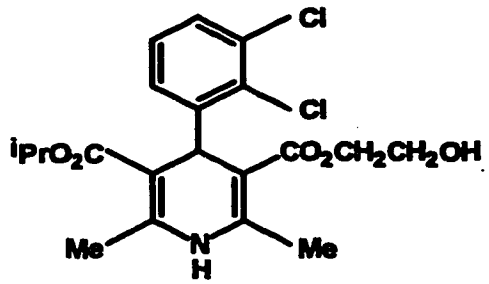
^{1}H NMR ($CDCl_3$): δ 7.33 (dd, J = 7.8 Hz, J = 1.4 Hz, 1H, aryl H-4), 7.28 (dd, J = 7.8 Hz, J = 1.4 Hz, 1H, aryl H-6), 7.11 (t, J = 7.8 Hz, 1H, aryl H-5), 5.74 (br s, 1H, N*H*), 5.48 (s, 1H, H-4), 4.17 (m, 2H, COOC*H*$_2$), 3.77 (t, J = 4.5 Hz, 2H, C*H*$_2$OH), 3.64 (s, 3H, OC*H*$_3$), 2.36 and 2.33 (two s, 3H each, C-2 and C-6 C*H*$_3$), 2.03 (br s, 1H, O*H*, exchanges with D_2O).

IR (KBr): 3336 (br) (NH) and (OH), 1688 (C=O) cm^{-1}.

Anal. Calcd. for $C_{18}H_{19}Cl_2NO_5$: C, 54.01; H, 4.78; N, 3.50.

Found: C, 54.01; H, 4.73; N, 3.46.

4.4.3.3.0.0. 3-(2-Hydroxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (103)



(103)

Compound (**103**) was prepared according to Procedure C by condensation of 2,3-dichlorobenzaldehyde (2.28 g, 13 mmol), 2-hydroxyethyl acetoacetate (**160**) (1.90 g, 13 mmol) and isopropyl 3-aminocrotonate (**74**) (1.86 g, 13 mmol). The product (**103**) was purified by elution from a silica gel column with ethyl acetate-hexane (1:1, v/v) as eluent. The product was recrystallized from CH_2Cl_2-hexane as a light yellow crystalline solid. (2.2 g, 39.3%); mp 150 - 151°C.

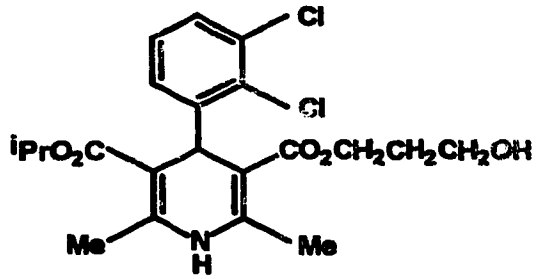
^{1}H NMR ($CDCl_3$): δ 7.34 (dd, J = 7.8 Hz, J = 1.6 Hz, 1H, aryl H-4), 7.28 (dd, J = 7.8 Hz, J = 1.6 Hz, 1H, aryl H-6), 7.10 (t, J = 7.8 Hz, 1H, aryl H-5), 5.72 (br s, 1H, N*H*), 5.44 (s, 1H, H-4), 5.0 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.17 (t, J = 4.2 Hz, 2H, C*H*$_2$OH), 3.76 (t, J = 4.2 Hz, 2H, COOC*H*$_2$), 2.33 and 2.31 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.7 - 2.1 (br s, 1H, O*H*, exchanges with D_2O), 1.27 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.07 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (film): 3175 - 3800 (br) (OH), 3338 (NH), 1679 (C=O) cm^{-1}.

Anal. Calcd. for $C_{20}H_{23}Cl_2NO_5.1/4\ H_2O$: C, 55.50; H, 5.47; N, 3.24.

Found: C, 55.59; H, 5.29; N, 3.22.

4.4.3.4.0.0. 3-(3-Hydroxypropyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (104)



(104)

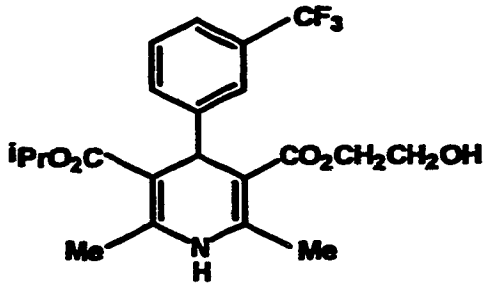
The title compound was synthesized according to Procedure C by condensation of 2,3-dichlorobenzaldehyde (1.64 g, 9.37 mmol), 3-hydroxypropyl acetoacetate (**163**) (1.5 g, 9.37 mmol) and isopropyl 3-aminocrotonate (**74**) (1.35 g, 9.37 mmol). The product was purified by elution from a silica gel column with ethyl acetate-hexane as eluent (1:1, v/v) to afford (**104**) as a pale yellow oil (1.88 g, 45 %).

^{1}H NMR ($CDCl_3$): δ 7.31 (dd, J = 7.8 Hz, J = 1.4 Hz, 1H, aryl H-4), 7.26 (dd, J = 7.8 Hz, J = 1.4 Hz, 1H, aryl H-6), 7.08 (t, J = 7.8 Hz, 1H, aryl H-5), 5.72 (br s, 1H, N*H*), 5.42 (s, 1H, H-4), 4.98 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.14 - 4.19 and 4.20 - 4.34 (two m, 2H total, COOC*H*$_2$). 3.28 - 3.32 and 3.39 - 3.44 (two m, 2H total, C*H*$_2$OH), 2.31 (s, 6H, C-2 and C-6 C*H*$_3$), 1.6 - 2.1 (m, 3H, $CH_2CH_2CH_2$ and O*H*, exchanges with D_2O), 1.25 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.04 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (film): 3030 - 3770 (br) (OH), 3415 (NH), 1723 (C=O) cm^{-1}.

Compound (**104**) was used as an immediately for the synthesis of (**101**).

4.4.3.5.0.c. 3-(2-Hydroxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-trifluoromethylphenyl)-3,5-pyridinedicarboxylate (110)



(110)

Compound (110) was prepared according to the method described in Procedure C by condensation of 3-trifluoromethylbenzaldehyde (3.48 g, 20 mmol), 2-hydroxyethyl acetoacetate (160) (2.92 g, 20 mmol) and isopropyl 3-aminocrotonate (74) (2.86 g, 20 mmol). The reaction product was purified by silica gel column chromatography with ethyl acetate-hexane as eluent (1:1, v/v) to afford (110) which was recrystallized from ether-hexane (4.8 g, 56%); mp 121 - 122°C.

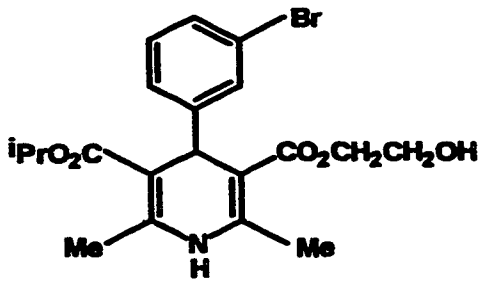
^{1}H NMR ($CDCl_3$): δ 7.55 (s, 1H, aryl H-2), 7.49 (d, J = 7.5 Hz, 1H, aryl H-4), 7.41 (d, J = 7.5 Hz, 1H, aryl H-6), 7.35 (t, J = 7.5 Hz, 1H, aryl H-5), 5.67 (br s, 1H, N*H*), 5.02 (s, 1H, H-4), 4.96 (septet, J= 6.2 Hz, 1H, C*H*Me$_2$), 4.21 - 4.28 and 4.08 - 4.15 (two m, 2H total, COOC*H*$_2$), 3.74 (br s, 2H, C*H*$_2$OH), 2.38 and 2.35 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.76 (br s, 1H, O*H*), 1.26 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.10 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3141 - 3665 (br) (OH), 3342 (NH), 1684 (C=O), 1109 - 1209 (C-F) cm^{-1}.

Anal. Calcd. for $C_{21}H_{24}F_3NO_5$: C, 59.01; H, 5.66; N, 3.28.

Found: C, 58.77; H, 5.53; N, 3.27.

4.4.3.6.0.0. 3-(2-Hydroxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-bromophenyl)-3,5-pyridinedicarboxylate (111)



(111)

The title compound (111) was prepared according to Procedure C by condensation of 3-bromobenzaldehyde (5.55 g, 30 mmol), 2-hydroxyethyl acetoacetate (160) (4.38 g, 30 mmol) and isopropyl 3-aminocrotonate (74) (4.30 g, 30 mmol). The reaction product was purified by elution from a silica gel column using ethyl acetate-hexane (1:1, v/v) as eluent. The product obtained was recrystallized from ether-hexane to afford (111) as a pale yellow crystalline solid (9.14 g, 69.5%); mp 115 -116°C.

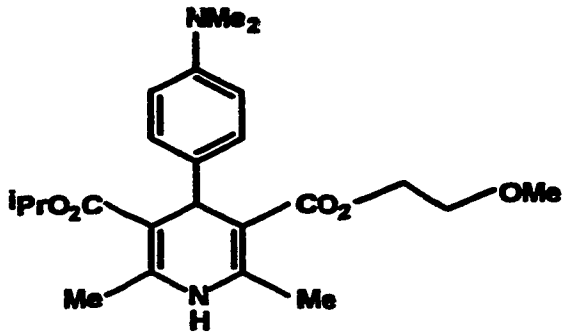
^{1}H NMR ($CDCl_3$): δ 7.41 (d, J = 1.7 Hz, 1H, aryl H-2), 7.20 - 7.28 (m, 2H, aryi H-4 and H-6), 7.08 (t, J = 7.8 Hz, 1H, aryl H-5), 5.69 (br s, 1H, N*H*), 4.95 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.91 (s, 1H, H-4), 4.22 - 4.29 and 4.04 - 4.11 (two m, 1H each, COOC*H*$_2$), 3.68 - 3.78 (m, 2H, C*H*$_2$OH), 2.35 and 2.31 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.78 (t, J = 6.1 Hz, 1H, O*H*), 1.25 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.13 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3734 (NH), 3390 (br) (OH), 1681 (C=O) cm^{-1}.

Anal. Calcd. for $C_{20}H_{24}BrNO_5$: C, 54.80; H, 5.52; N, 3.20; Br, 18.23.

Found: C, 54.37; H, 5.48; N, 3.10; Br, 17.96.

4.4.3.7.0.0. 3-(2-Methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(4-dimethylaminophenyl)-3,5-pyridinedicarboxylate (150)



(150)

Compound (150) was prepared according to Procedure C by condensation of 4-dimethylaminobenzaldehyde (7.46 g, 50 mmol), 2-methoxyethyl acetoacetate (73) (8.00 g, 50 mmol) and isopropyl 3-aminocrotonate (7.16 g, 50 mmol). The reaction was performed by warming the reaction mixture in 2-methoxyethanol (50 ml) at 100°C for 48 hours. The product was purified by elution from a silica gel column using ethyl acetate-hexane (1:1, v/v), and the solid obtained was recrystallized from ethyl acetate-hexane to afford (150) as a pale yellow crystalline solid (5.21 g, 25%); mp 139.5 - 141°C.

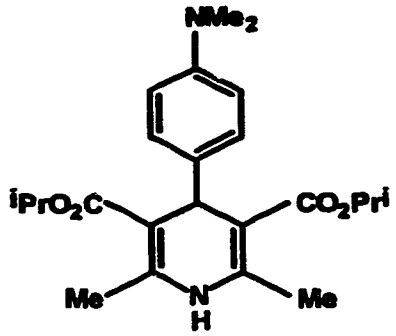
^{1}H NMR: δ 7.16 (d, J = 8.7 Hz, 2H, aryl H-2, H-6), 6.61 (d, J = 8.7 Hz, 2H, aryl H-3, H-5), 5.56 (br s, 1H, N*H*), 4.95 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.90 (s, 1H, H-4), 4.19 (t, J = 4.9 Hz, 2H, COOC*H*$_2$), 3.58 (t, J = 4.9 Hz, 2H, C*H*$_2$OMe), 3.38 (s, 3H, OC*H*$_3$), 2.89 (s, 6H, NC*H*$_3$), 2.33 and 2.32 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.25 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.15 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3351 (NH), 1695, 1651 (C=O), 1111 (C-O-C) cm^{-1}.

Anal. Calcd for $C_{23}H_{32}N_2O_5$: C, 66.32; H, 7.74; N, 6.73.

Found: C, 66.61; H, 7.82; N, 6.77.

4.4.3.8.0.0. 3,5-Diisopropyl 1,4-dihydro-2,6-dimethyl-4-(4-dimethylaminophenyl)-3,5-pyridinedicarboxylate (151)



(151)

The synthetic procedure was similar to that employed for the preparation of (150) which involved the condensation of 4-dimethylaminobenzaldehyde (4.48 g, 30 mmol), isopropyl 3-aminocrotonate (74) (4.30 g, 30 mmol) and isopropyl acetoacetate (161) (4.37 g, 30 mmol). The light yellow crystalline product obtained was recrystallized from ethyl acetate-hexane to yield (151) (2.30 g, 19%); mp 135 - 136°C.

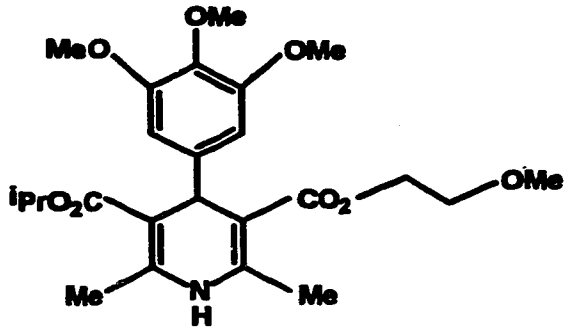
^{1}H NMR ($CDCl_3$): 7.16 (d, J = 7.8 Hz, 2H, aryl H-2, H-6), 6.62 (d, J = 7.8 Hz, 2H, aryl H-3, H-5), 5.47 (br s, 1H, N*H*), 4.97 (septet, J = 6.0 Hz, 2H, C*H*Me$_2$), 4.88 (s, 1H, H-4), 2.90 (s, 6H, NC*H*$_3$), 2.33 (s, 6H, C-2 and C-6 C*H*$_3$), 1.26 (d, J = 6.0 Hz, 6H, CHC*H*$_3$), 1.16 (d, J = 6.0 Hz, 6H, CHC*H*$_3$).

IR (KBr): 3394 (NH), 1691 (C=O) cm^{-1}.

Anal. Calcd for $C_{23}H_{32}N_2O_4$: C, 68.97; H, 8.05; N, 6.99.

Found: C, 68.67; H, 8.16; N, 6.79.

4.4.3.9.0.0. 3-(2-Methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3,4,5-trimethoxyphenyl)-3,5-pyridinedicarboxylate (154)



(154)

The title compound compound (**154**) was prepared according to Procedure C by condensation of 3,4,5-trimethoxybenzaldehyde (2.29 g, 12 mmol), isopropyl 3-amino-crotonate (**74**) (1.67 g, 12 mmol) and 2-methoxyethyl acetoacetate (**73**) (1.87 g, 12 mmol). The product was isolated by elution from a silica gel column using ethyl acetate-hexane (1:1, v/v) as eluent and the solid obtained was recrystallized from ethyl acetate-hexane to afford (**154**) as pale yellow crystals (2.35 g, 42%); mp 119 - 120°C.

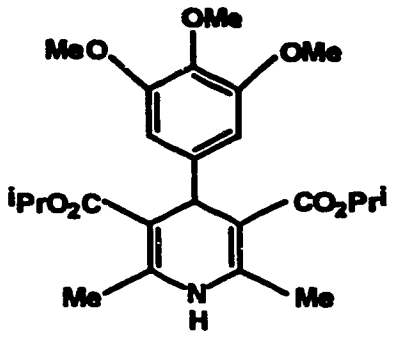
^{1}H NMR ($CDCl_3$) δ 6.54 (s, 2H, aryl H-2, H-6), 5.57 (br s, 1H, N*H*), 4.97 - 5.04 (m, 2H, C*H*Me$_2$, H-4), 4.23 (t, J = 4.8 Hz, 2H, COOC*H*$_2$), 3.81, 3.80 (two s, 9H total, aryl OC*H*$_3$), 3.59 (t, J = 4.8 Hz, 2H, C*H*$_2$OMe), 3.35 (s, 3H, $CH_2CH_2OCH_3$), 2.45 (s, 6H, C-2 and C-6 C*H*$_3$), 1.26 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.16 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3335 (NH), 1689 (C=O), 1111 (C-O-C) cm^{-1}.

Anal. Calcd for $C_{24}H_{33}NO_8$: C, 62.19; H, 7.18; N, 3.02.

Found: C, 62.18; H, 7.36; N, 3.04.

4.4.3.10.0.0. 3,5-Diisopropyl 1,4-dihydro-2,6-dimethyl-4-(3,4,5-trimethoxyphenyl)-3,5-pyridinedicarboxylate (155)



(155)

The title compound (**155**) was synthesized according to Procedure C by condensation of 3,4,5-trimethoxybenzaldehyde (2.72 g, 13.9 mmol), isopropyl 3-aminocrotonate (**74**) (2.00 g, 13.9 mmol) and isopropyl acetoacetate (**161**). The product was isolated by elution from a silica gel column using ethyl acetate-hexane (1:1, v/v) as eluent. Recrystallization of the product from ethyl acetate-hexane afforded (**155**) as a pale yellow crystalline solid (1.26 g, 20.3%); mp 161 -162°C.

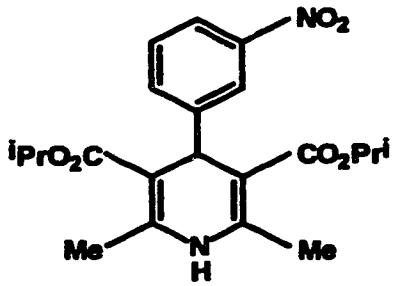
^{1}H NMR ($CDCl_3$): δ 6.53 (s, 2H, aryl H-2, H-6), 5.53 (br s, 1H, N*H*), 4.95 - 5.04 (m, 3H, C*H*Me_2 and H-4), 3.81, 3.79 (two s, 9H total, OCH_3), 2.35 (s, 6H, C-2 and C-6 CH_3), 1.26 (d, J = 6.3 Hz, 6H, CHCH_3), 1.18 (d, J = 6.3 Hz, 6H, CHCH_3).

IR (KBr): 3361 (NH), 1695 (C=O) cm^{-1}.

Anal. Calcd for $C_{24}H_{33}NO_7$: C, 64.41; H, 7.43; N, 3.13.

Found: C, 64.53; H, 7.51; N, 3.17.

4.4.3.11.0.0. 3,5-Diisopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (156)



(156)

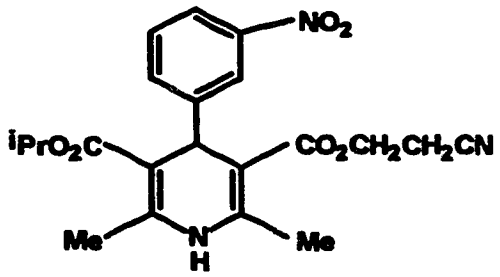
Compound (156) was prepared according to Procedure C by condensation of 3-nitrobenzaldehyde (4.53 g, 30 mmol), isopropyl 3-aminocrotonate (74) (4.30 g, 30 mmol) and isopropyl acetoacetate (161) (4.33 g, 30 mmol). The product was isolated by elution from a silica gel column using ethyl acetate-hexane (1:2, v/v) as eluent, and the product was recrystallized from CH_2Cl_2-hexane to afford (156) as a pale yellow solid (7.71 g, 64%); mp 130 - 131°C.

^{1}H NMR ($CDCl_3$): δ 8.14 (t, J = 1.2 Hz, 1H, aryl H-2), 8.00 (ddd, J = 8.0 Hz, J = 1.2 Hz, J = 1.2 Hz, 1H, aryl H-4), 7.65 (ddd, J = 8.0 Hz, J = 1.2 Hz, J = 1.2 Hz, 1H, aryl H-5), 7.37 (t, J = 8.0 Hz, 1H, aryl H-5), 5.68 (br s, 1H, N*H*), 5.06 (s, 1H, H-4), 4.95 (septet, J = 6.3 Hz, 2H, C*H*Me$_2$), 2.36 (s, 6H, C-2 and C-6 C*H*$_3$), 1.26 (d, J = 6.3 Hz, 6H, CHC*H*$_3$), 1.10 (d, J = 6.3 Hz, 6H, CHC*H*$_3$).

IR (KBr): 3362 (NH), 1652, 1701 (C=O), 1531, 1348 (NO_2) cm^{-1}.

Compound (156) was used as an intermediate for the synthesis of (153).

4.4.3.12.0.0. 3-(2-Cyanoethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (164)



(164)

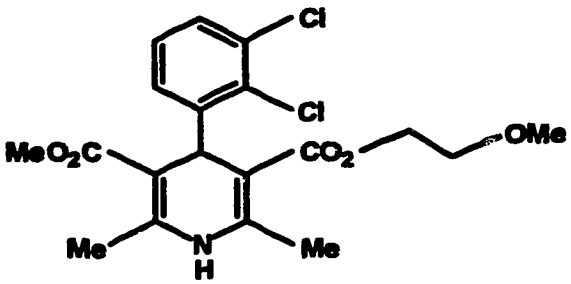
The title compound (**164**) was synthesized according to Procedure C by condensation of 3-nitrobenzaldehyde (4.49 g, 30 mmol), 2-cyanoethyl 3-aminocrotonate (**89**) (4.59 g, 30 mmol) and isopropyl acetoacetate (**161**) (4.29 g, 30 mmol). The product was isolated by elution from a silica gel column using ethyl acetate-hexane (1:2, v/v) as eluent. Recrystallization from CH_2Cl_2-hexane yielded (**164**) as a yellow crystalline solid (3.14 g, 26%); mp 132 -136°C.

^{1}H NMR ($CDCl_3$): δ 8.13 (s, 1H, aryl H-2), 8.03 (d, J = 7.7 Hz, 1H, aryl H-4), 7.68 (d, J = 7.7 Hz, 1H, aryl H-6), 7.41 (t, J = 7.7 Hz, 1H, aryl H-5), 5.76 (br s, 1H, N*H*), 5.09 (s, 1H, H-4), 4.97 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.22 - 4.31 (m, 2H, COOC*H*$_2$), 2.65 (t, J = 6.1 Hz, 2H, C*H*$_2$CN), 2.40 and 2.38 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.28 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.12 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3378 (NH), 2254 (CN), 1647, 1696 (C=O), 1532, 1352 (NO_2).

Compound (**164**) was used as a precursor for the synthesis of (**142**).

4.4.3.13.0.0. 3-(2-Methoxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (165).



(165)

The title compound **(165)** was prepared according to Procedure C by condensation of 2,3-dichlorobenzaldehyde (1.75 g, 10 mmol), methyl 3-aminocrotonate (1.15 g, 10 mmol) and 2-methoxyethyl acetoacetate (1.60 g, 10 mmol). The reaction product was purified by silica gel column chromatography using ethyl acetate-hexane (1:1, v/v) as eluent to afford **(165)** which was recrystallized from ethyl acetate-hexane (0.86 g, 20.8%); mp 174 - 176°C.

^{1}H NMR ($CDCl_3$): δ 7.31 (dd, J = 7.8 Hz, J = 1.4 Hz, 1H, aryl H-4), 7.25 (dd, J = 7.8 Hz, J = 1.4 Hz, 1H, aryl H-6), 7.07 (t, J = 7.8 Hz, 1H, aryl H-5), 5.64 (br s, 1H, N*H*), 5.48 (s, 1H, H-4), 4.17 (t, J = 4.9 Hz, 2H, $COOCH_2CH_2$), 3.61 (s, 3H, $COOCH_3$), 3.54 (m, 2H, CH_2CH_2OMe), 3.31 (s, 3H, $CH_2CH_2OCH_3$), 2.33 and 2.32 (two s, 3H each, C-2 and C-6 CH_3).

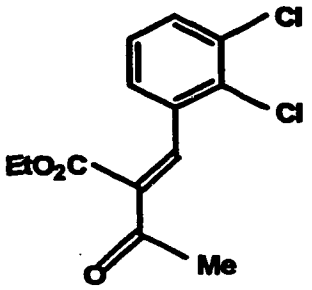
IR (KBr): 3287, 3238 (NH), 1688, (C=O), 1092 (C-O-C) cm^{-1}.

Compound **(165)** was used as an internal standard for the quantitative HPLC analyses of **(118)**.

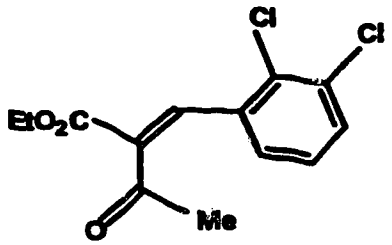
4.4.4.0.0.0. Synthesis of 3-ethyl 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (Felodipine) (39)

4.4.4.1.0.0. Synthesis of ethyl 2-(2,3-dichlorobenzylidene)acetoacetate (80)

General procedure for the synthesis of alkyl 2-(2,3-dichlorobenzylidene)acetoacetates (Procedure D)



(*Z*)-(80)



(*E*)-(80)

Alkyl 2-(2,3-dichlorobenzylidene)acetoacetates were prepared using a general procedure described by Müller.[225] A solution containing 2,3-dichlorobenzaldehyde (6.13 g, 0.035 mol), ethyl acetoacetate (4.56 g, 0.035 mol), piperidine (0.17 g. 2 mmol) and glacial acetic acid (0.6 g, 10 mmol) in benzene (50 ml) was refluxed using a Dean-Stark water separator for 6 hours at which time water was no longer being generated in the reaction. Removal of solvent *in vacuo* gave a residue which was dissolved in CH_2Cl_2 (50 ml) and consecutively washed with Na_2CO_3 solution (10% w/v, 3x10 ml) and water (3 x 10 ml). The organic layer was dried (Na_2SO_4), the solvent was removed, and the residue obtained was purified by silica gel column chromatography using EtOAc-hexane (1:8, v/v) as eluent. The first component eluted was the (*E*)-isomer (4.38 g, 44%). Continued elution afforded the (*Z*)-isomer (4.63 g, 46%). Both isomers were yellow oils after evaporation of the solvent.

The assignment of the (*E*)- and (*Z*)- isomer is based on the ^1H NMR spectra. The methyl protons (CH_3CO) of the (*E*)- isomer are *cis*- to the aromatic ring and are located in the shielding zone of the aryl ring, and hence resonate at a higher field (δ = 2.22) whereas, the methyl protons of the (*Z*)-isomer are not influenced by the *trans*-aromatic ring and hence resonate at lower field (δ = 2.45).

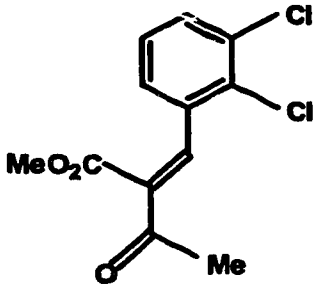
^{1}H NMR ($CDCl_3$) for (*Z*)-isomer: δ 7.80 (s, 1H, vinyl H), 7.48 (dd, J = 7.9 Hz, J = 1.4 Hz, 1H, aryl H-4), 7.32 (dd, J = 7.9 Hz, J = 1.4 Hz, 1H, aryl H-6), 7.19 (t, J = 7.9 Hz, 1H, aryl H-5), 4.20 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 2.45 (s, 3H, CH_3CO), 1.14 (t, J = 7.1 Hz, 3H, OCH_2CH_3).

^{1}H NMR ($CDCl_3$) for (*E*)-isomer: δ 7.88 (s, 1H, vinyl H), 7.48 (dd, J = 7.9 Hz, J = 1.4 Hz, 1H, aryl H-4), 7.32 (dd, J = 7.9 Hz, J = 1.4 Hz, 1H, aryl H-6), 7.19 (t, J = 7.9 Hz, 1H, aryl H-5), 4.31 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 2.22 (s, 3H, CH_3CO), 1.34 (t, J = 7.1 Hz, 3H, OCH_2CH_3).

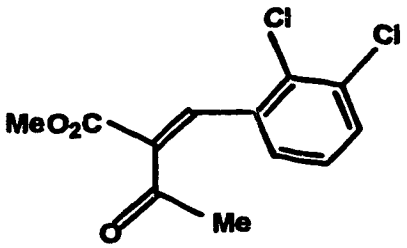
IR (film): 1730 (C=O, ester), 1708 (C=O, ketone), 1627 (C=C) cm^{-1}.

Compound (**80**), both (*Z*)- and (*E*)-isomers, were used as intermediates for the synthesis of (**90**).

4.4.4.1.1.0. Methyl 2-(2,3-dichlorobenzylidene)acetoacetate (87)



(*Z*)-(87)



(*E*)-(87)

The title compounds (*Z*)-(87) and (*E*)-(87) were prepared according to procedure D by condensing 2,3-dichlorobenzaldehyde (17.5 g, 100 mmol), methyl acetoacetate (11.6 g, 100 mmol), piperidine (0.34 g, 4 mmol) and glacial acetic acid (1.2 g, 20 mmol). The compound was isolated by elution from a silica gel column using ethyl acetate-hexane (1:6, v/v). The first component eluted was the (*Z*)-isomer (11.7 g, 43%). Continued elution afforded the (*E*)-isomer (12.63 g, 46%). Both isomers were recrystallized separately from ether-hexane to afford colourless crystals, mp for the (*E*) isomer : 51 - 53°C; mp for the (*Z*)-isomer 81 - 82°C.

The rationale used for the assignment of the (*E*)- and (*Z*)-isomers was similar to that used for (**80**).

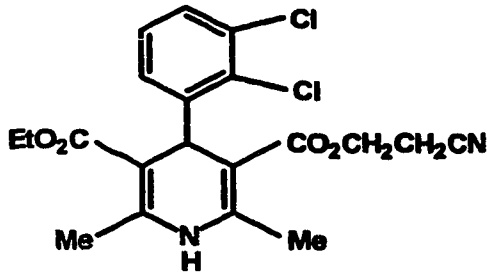
^{1}H NMR ($CDCl_3$) for the (*E*)-isomer: δ 7.94 (s, 1H, vinyl-H), 7.48 (dd, J = 7.8 Hz, J = 1.7 Hz, 1H, aryl H-4), 7.27 (dd, J = 7.8 Hz, J = 1.7 Hz, 1H, aryl H-6), 7.28 (t, J = 7.8 Hz, 1H, aryl H-5), 3.88 (s, 3H, $COOCH_3$), 2.23 (s, 3H, $COCH_3$).

^{1}H NMR ($CDCl_3$) for the (*Z*)-isomer: δ 7.81 (s, 1H, vinyl-H), 7.48 (dd, J = 7.8 Hz, J = 1.7 Hz, 1H, aryl H-4), 7.27 (dd, J = 7.8 Hz, J = 1.7 Hz, 1H, aryl H-6), 7.28 (t, J = 7.8 Hz, 1H, aryl H-5), 3.71 (s, 3H, $COOCH_3$), 2.44 (s, 3H, $COCH_3$).

IR (KBr): 1723 (C=O, ester) 1716 (C=O, ketone), 1631 (C=C), 1237(C-O).

Compound (**87**), both (*E*)- and (*Z*)- isomers were used as intermediates for the synthesis of (**128**).

4.4.4.2.0.0. Synthesis of 3-(2-cyanoethyl) 5-ethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (90)



(90)

A mixture of the (*E*)- and (*Z*)-isomers of ethyl 2-(2,3-dichlorobenzylidene)acetate (**80**) (5.74 g, 20 mmol) and 2-cyanoethyl 3-aminocrotonate (**89**) (3.08 g, 20 mmol) in dry *t*-BuOH (25 ml) was stirred at 25°C for 96 hours. Removal of the solvent *in vacuo* and separation of the residue obtained by silica gel column chromatography using EtOAc-hexane (3:7, v/v) as eluent yielded (**90**) as a yellow oil (6.04 g, 71%). Further elution afforded the symmetrical 3,5-(2-cyanoethyl) DHP (**91**) as a pale yellow solid (0.46 g, 6%) .

^{1}H NMR ($CDCl_3$): δ 7.32 (dd, J = 7.7 Hz, J = 1.6 Hz, 1H, aryl H-4), 7.27 (dd, J = 7.7 Hz, J = 1.6 Hz, 1H, aryl H-6), 7.10 (t, J = 7.7 Hz, 1H, aryl H-5), 5.86 (br s, 1H, N*H*), 5.45 (s, 1H, H-4), 4.24 (t, J = 6.4 Hz, 2H, $CO_2CH_2CH_2$), 4.09 (q, J = 7.0 Hz, 2H,

CH_2CH_3), 2.66 (t, J = 6.4 Hz, 2H, CH_2CN), 2.33 and 2.31 (two s, 3H each, C-2 and C-6 CH_3), 1.19 (t, J = 7.0 Hz, 3H, CH_2CH_3).

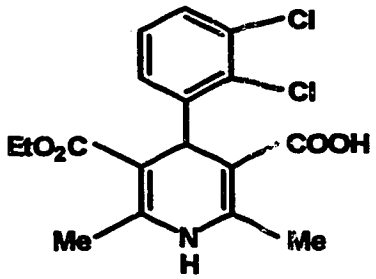
IR (neat): 3344 (NH), 2256 (CN), 1719 (C=O) cm^{-1}.

Anal. Calcd. for $C_{20}H_{20}Cl_2N_2O_4$: C, 56.75; H, 4.76; N, 6.62.

Found: C, 56.55; H, 4.66; N, 6.45.

4.4.4.3.0.0. Synthesis of ethyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (92)

General synthesis for alkyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (Procedure E)



(92)

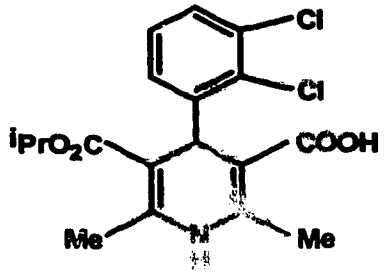
1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 4.41 g, 29 mmol) was added to a solution of (**90**) in MeOH (50 ml) and the reaction was allowed to proceed at 25°C for 48 hours with stirring prior to adjustment of the pH to 1 using 2N HCl. The resulting yellow precipitate was filtered, washed successively with water (3 x 35 ml) and ether (3 x 25 ml), and the pale yellow product was dried *in vacuo* (2.6 g, 72.7%); m.p. 184 - 185°C (dec).

^1H NMR (DMSO-d_6): δ 11.65 (br s, 1H, COO*H*, exchanges with D_2O), 8.79 (s, 1H, N*H*, exchanges with D_2O), 7.38 (dd, J = 7.7 Hz, J = 2.1 Hz, 1H, aryl H-4), 7.29 (dd, J = 7.7 Hz, J = 2.1 Hz, 1H, aryl H-6), 7.23 (t, J = 7.7 Hz, 1H, aryl H-5), 5.29 (s, 1H, H-4), 3.94 (q, J = 7.0 Hz, 2H, CH_2CH_3), 2.22 (s, 6H, C-2 and C-6 CH_3), 1.07 (t, J = 7.0 Hz, 3H, CH_2CH_3).

IR (KBr): 2900 - 3600 (COOH), 3397 (NH), 1680 (C=O, acid), 1658 (C=O, ester), 1220 (C-O, acid) cm^{-1}.

Anal. Calcd. for $C_{17}H_{17}Cl_2NO_4$: C, 55.15; H, 4.63; N, 3.78.
Found: C, 55.28; H, 4.42; N, 3.80.

4.4.4.3.1.0. Isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (94)



(94)

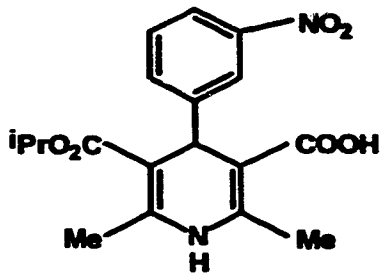
The title compound (**94**) was prepared according to the method described in Procedure E by reaction of (**96**) (6.79 g, 15.53 mmol) with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 7.09 g, 46.59 mmol) to afford (**94**) as a pale yellow solid (4.78 g, 80%); mp 196°C (dec).

^{1}H NMR (DMSO-d_6): δ 8.74 (br s, 1H, N*H*), 7.37 (d, J = 7.3 Hz, 1H, aryl H-4), 7.20 - 7.29 (m, 2H, aryl H-6 and H-5), 5.26 (s, 1H, H-4), 4.81 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 2.21 and 2.19 (two s, 3H each, C-2 and C-6 CH_3), 1.16 (d, J = 6.2 Hz, 3H, CHCH_3), 0.93 (d, J = 6.2 Hz, 3H, CHCH_3).

IR (KBr): 2092 - 3666 (br) (COOH), 3344 (NH), 1671 (C=O, both acid and ester), 1229 (C-O, acid) cm^{-1}.

Compound (**94**) was used immediately for the synthesis of (**98**).

4.4.4.3.2.0. Isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylate (142)



(142)

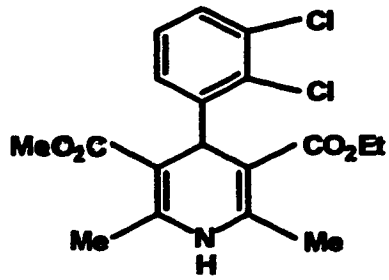
The title compound was prepared according to the method described in Procedure E by reaction of **(164)** (3.08 g, 7.45 mmol) with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 3.40 g, 22.35 mmol) to afford **(142)** as a pale yellow solid (1.77 g, 66%); mp 165 - 169°C(dec).

^{1}H NMR (DMSO-d_6): δ 11.82 (br s, 1H, COO*H*, exchanges with D_2O), 8.89 (s, 1H, N*H*), 7.99 - 8.02 (m, 2H, aryl H-4 and H-2), 7.52 - 7.61 (m, 2H, aryl H-6, H-5), 4.94 (s, 1H, H-4), 4.82 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 2.28 and 2.27 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.19 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.04 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (KBr): 2154 - 3846 (br) (COOH), 3364 (NH), 1699 (C=O, acid), 1679 (C=O, ester), 1528, 1349 (NO_2) cm^{-1}.

Compound **(142)** was used immediately for the synthesis of **(144)**.

4.4.4.4.0.0. Synthesis of 3-ethyl 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (39)



(39)

Iodomethane (0 84 ml, 13.5 mmol) was added to a solution of (92) (1.0 g, 2.7 mmol) in dry DMF (20 ml) and K_2CO_3 (1.12 g, 8 mmol) and the reaction was allowed to proceed at 25°C for 24 hours with stirring. The reaction mixture was filtered and the solvent from the filtrate was removed *in vacuo* to remove as much DMF as was possible. The residue obtained was dissolved in CH_2Cl_2 (20 ml), washed with water (3 x 25 ml) and this CH_2Cl_2 solution was dried (Na_2SO_4). Removal of the solvent *in vacuo* and purification of the product obtained by elution from a silica gel column using EtOAc-hexane (1:1, v/v) as eluent afforded (39) as colorless needles after recrystallization from CH_2Cl_2 and then from diisopropyl ether (0.73 g, 70%); mp 140 - 141°C, (lit.[196] mp 145° C).

^{1}H NMR ($CDCl_3$): δ 7.29 (dd, J = 7.7 Hz, J = 1.7 Hz, 1H, aryl H-4), 7.24 (dd, J = 7.7 Hz, J = 1.7 Hz, 1H, aryl H-6), 7.06 (t, J = 7.7 Hz, 1H, aryl H-5), 5.58 (br s, 1H, N*H*), 5.46 (s, 1H, H-4), 4.07, (q, J = 7.1 Hz, 2H, C*H*$_2$CH$_3$), 3.61 (s, 3H, OC*H*$_3$), 2.32 and 2.31 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.18 (t, J = 7.1 Hz, 3H, CH$_2$C*H*$_3$).

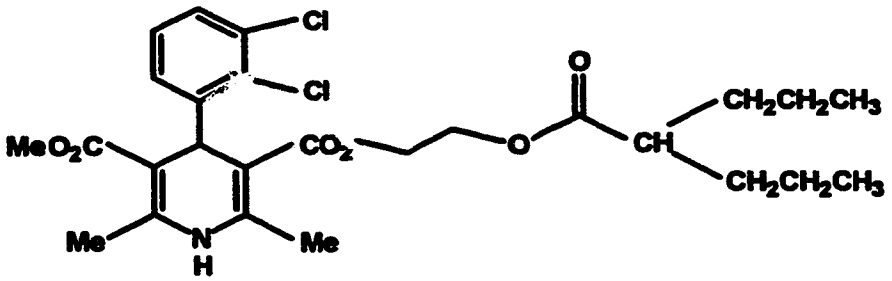
IR (KBr): 3362 (NH), 1700 (C=O) cm^{-1}.

Anal. Calcd. for $C_{18}H_{19}Cl_2NO_4$: C, 56.25; H, 4.98; N, 3.65.

Found: C, 56.55; H, 4.69; N, 3.59.

4.4.5.0.0.0. Syntheses of 3-(2-*n*-propylpentanoyloxyalkyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (99 - 101)

4.4.5.1.0.0. Synthesis of 3-[2-(2-*n*-propylpentanoyloxy)ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (99) (Procedure F)



(99)

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.537 g, 2.8 mmol) was added with stirring to a solution of (**102**) (1.0 g, 2.5 mmol), 2-propylpentanoic acid (valproic acid) (0.403 g, 2.8 mmol) and 4-dimethylaminopyridine (DMAP) (0.153 g, 1.4 mmol) in CH_2Cl_2 (20 ml) at 0°C and the reaction was allowed to proceed at 25°C for 16 hours. The reaction mixture was then washed consecutively with hydrochloric acid (0.5N) (3 x 15 ml), $NaHCO_3$ (10% w/v, 3 x 15 ml) and water (3 x 10 ml). The organic layer was dried (Na_2SO_4), the solvent was removed *in vacuo* and the residue obtained was eluted from a silica gel column using CH_2Cl_2-MeOH (97:3, v/v) as eluent to yield (**99**) as a yellowish oil after removal of the solvent (1.25 g, 95%).

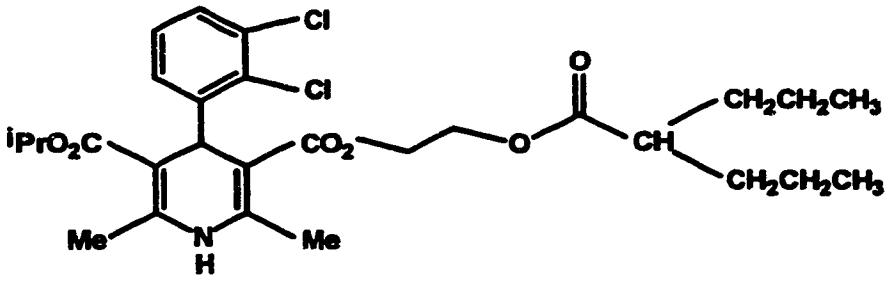
^{1}H NMR ($CDCl_3$): δ 7.30 (dd, J = 7.8 Hz, J = 1.6 Hz, 1H, aryl H-4), 7.25 (dd, J = 7.8 Hz, J = 1.6 Hz, 1H, aryl H-6), 7.07 (t, J = 7.8 Hz, 1H, aryl H-5), 5.67 (br s, 1H, N*H*), 5.47 (s, 1H, H-4), 4.15 - 4.30 (m, 4H, COOC*H*$_2$C*H*$_2$OCO), 3.61 (s, 3H, OC*H*$_3$), 2.32 - 2.38 (m, 7H, C*H*Pr^n and C-2, C-6 C*H*$_3$), 1.21 - 1.63 (m, 8H, CHC*H*$_2$C*H*$_2$CH$_3$), 0.85 - 0.91 (m, 6H, CHCH$_2$CH$_2$C*H*$_3$).

IR ($CHCl_3$): 3347 (NH), 1694 (C=O) cm^{-1}.

Anal. Calcd. for $C_{26}H_{33}Cl_2NO_6$: C, 59.32; H, 6.32; N, 2.66.

Found: C, 59.01; H, 6.35; N, 2.64.

4.4.5.1.1.0. Synthesis of 3-[2-(2-*n*-propylpentanoyloxy)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (100)



(100)

The title compound (**100**) was synthesized using the method described in Procedure F by reaction of (**103**) (1.00 g, 2.34 mmol), 2-propylpentanoic acid (0.375 g, 2.6 mmol), EDCI (0.498 g, 2.6 mmol) and DMAP (0.143 g, 1.3 mmol). The target

compound (**100**) was isolated by elution from a silica gel column using CH_2Cl_2-MeOH (97:3, v/v) as eluent to yield a yellow oil (1.17 g, 90.3%).

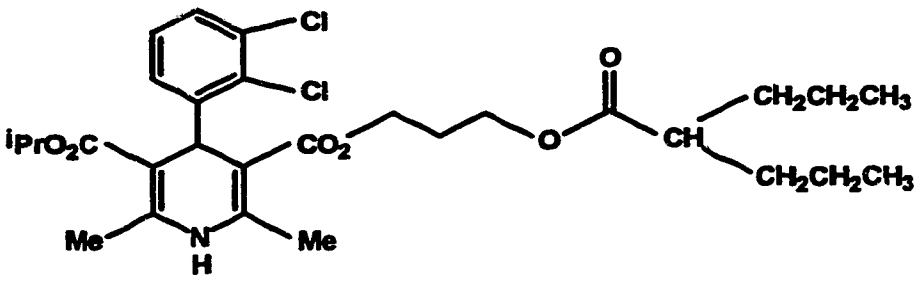
^{1}H NMR ($CDCl_3$): δ 7.33 (dd, J = 7.8 Hz, J = 1.6 Hz, 1H, aryl H-4), 7.24 (dd, J = 7.8 Hz, J = 1.6 Hz, 1H, aryl H-6), 7.07 (t, J = 7.8 Hz, 1H, aryl H-5), 5.64 (br s, 1H, N*H*), 5.43 (s, 1H, H-4), 4.97 (septet, J = 6.3 Hz, 1H, C*H*Me), 4.12 - 4.36 (m, 4H, COOC*H*$_2$C*H*$_2$OCO), 2.20 - 2.48 (m, 7H, C*H*Pr^n, C-2 and C-6 C*H*$_3$), 1.14 - 1.72 (m, 11H, CHC*H*$_3$, CHC*H*$_2$C*H*$_2$CH$_3$), 1.03 (d, J = 6.3 Hz, 3H, CHC*H*$_3$), 0.80 - 0.96 (m, 6H, $CH_2CH_2CH_3$).

IR ($CHCl_3$): 3356 (NH), 1688 (C=O) cm^{-1}.

Anal. Calcd. for $C_{28}H_{37}Cl_2NO_6$: C, 60.65; H, 6.73; N, 2.53.

Found: C, 60.56; H, 6.83; N, 2.53.

4.4.5.1.2.0. Synthesis of 3-[3-(2-*n*-propylpentanoyloxy)propyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (101)



(101)

Compound (**101**) was prepared using the method described in Procedure F by reaction of (**104**) (0.07 g, 0.158 mmol), 2-propylpentanoic acid (0.025 g, 0.174 mmol), EDCI (0.0334 g, 1.74 mmol) and DMAP (0.0106 g, 0.087 mmol). The residue obtained was purified by elution from a silica gel column using ethyl acetate-hexane (1:1 v/v) as eluent to yield (**101**) as a yellowish oil (0.0572 g, 64%).

^{1}H NMR ($CDCl_3$): δ 7.31 (dd, J = 7.8 Hz, J = 1.5 Hz, 1H, aryl H-4), 7.26 (dd, J = 7.8 Hz, J = 1.5 Hz, 1H, aryl H-6), 7.07 (t, J = 7.8 Hz, 1H, aryl H-5), 5.43 (br s, 1H, N*H*), 5.31 (s, 1H, H-4), 4.98 (septet, J = 6.3 Hz, 1H, C*H*Me), 4.12 (t, J = 6.4 Hz, 2H, COOC*H*$_2$), 4.03 (t, J = 6.4 Hz, 2H, C*H*$_2$OCO), 2.31 - 2.38 (m, 7H, C*H*Pr^n, C-2 and C-6

C*H*$_3$), 1.88 - 2.05 (m, 2H, COOCH$_2$C*H*$_2$CH$_2$OCO), 1.44 - 1.64 (m, 2H, CHC*H*$_2$CH$_2$CH$_3$), 1.31 - 1.42 (m, 2H, CHC*H*$_2$CH$_2$CH$_3$), 1.08 - 1.29 (m, 7H, CHC*H*$_3$ and two CHCH$_2$C*H*$_2$CH$_3$), 1.05 (d, J = 6.3 Hz, 3H, CHC*H*$_3$), 0.89 (t, J = 7.2 Hz, 6H, two CH$_2$C*H*$_3$).

IR (film): 3354 (NH), 1732 (CO) cm^{-1}.

Anal. Calcd. for $C_{29}H_{39}Cl_2NO_6.1/2H_2O$: C, 60.31; H, 6.98; N, 2.43.

Found: C, 60.24; H, 6.62; N, 2.55.

4.4.5.2.0. Synthesis of 3-(2-*n*-propylpentanoyloxymethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (98)

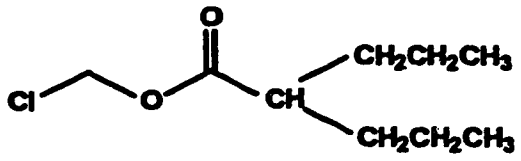
Chloromethylchlorosulphate (**166**) and chloromethyl valproate (**97**) precursors for the synthesis of (**98**), were synthesized by methods described by Binderup and Hansen.[226]

4.4.5.2.1.0. Synthesis of chloromethylchlorosulphate ($ClSO_3CH_2Cl$) (166)

Chlorosulphonic acid (40 ml, 600 mmol) was added carefully with stirring to bromochloromethane (20 ml, 300 mol) at ice-bath temperature. This mixture was heated at reflux for 3 hours during which bromine is liberated in the reaction. After cooling to 25°C, the reaction mixture was carefully poured onto ice. This mixture was extracted with CH_2Cl_2 (3 x 30 ml) and dried (Na_2SO_4) before removal of the solvent *in vacuo* to afford a yellow liquid. Distillation of this product *in vacuo* afforded (**166**) as a colourless liquid [bp 32 -34°C (3 mm Hg)] [Lit.[226] bp 45 - 50°C (9 - 10 mm Hg)]. (4.58 g, 9.3%).

^{1}H NMR ($CDCl_3$): δ 5.98 (s, C*H*$_2$).

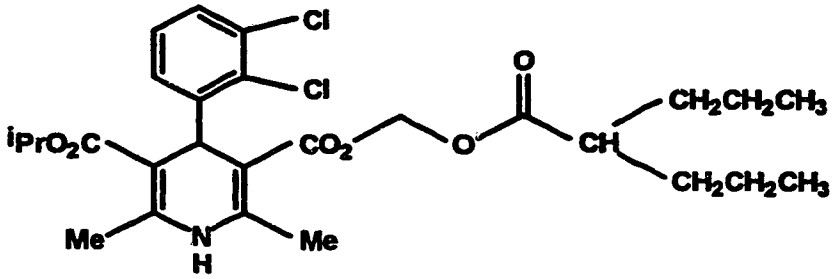
4.4.5.2.2.0. Synthesis of chloromethyl valproate (97)



(**97**)

To a solution of 2-propylpentanoic acid (valproic acid) (0.874 g, 6.06 mmol), $NaHCO_3$ (2.0 g, 23.8 mmol) and tetrabutylammonium hydrogen sulphate (0.2 g, 0.59 mmol) in water (40 ml) was added a solution of chloromethylchlorosulphate (166) (1.2 g, 7.27 mmol) in CH_2Cl_2 (40 ml). The reaction was allowed to proceed at 25°C for 45 min with stirring. The organic phase was separated, washed with water (3 x 30 ml), dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield a light yellow liquid. Distillation of this product yielded (97) as a colourless liquid [bp 74°C (3 mm Hg)] (1.67 g, 78.8%).
^1H NMR ($CDCl_3$): δ 5.71 (s, 2H, CH_2Cl), 2.43 (m, 1H, $CHPr^n$), 1.24 - 1.69 (m, 8H, two $CH_2CH_2CH_3$), 0.89 (t, J = 7.3 Hz, 6H, two $CH_2CH_2CH_3$)
IR (film): 1764 (C=O), 1113 (C-O).
The product (97) was used immediately in the subsequent reaction.

4.4.5.2.3.0. Synthesis of 3-(2-*n*-propylpentanoyloxymethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (98)



(98)

A solution of (94) (1.20 g, 3.12 mmol), potassium carbonate (1.29 g, 9.33 mmol), chloromethyl valproate (97) (0.65 g, 3.37 mmol) in DMF (30 ml) was stirred at 25°C for 16 hours. The reaction mixture was then filtered and the residue was washed with CH_2Cl_2 (3 x 15 ml). The combined organic fractions were then evaporated *in vacuo* so that as much DMF as much as possible was removed. The residue obtained was then dissolved in ethyl acetate (30 ml), this solution was washed with water (3 x 25 ml) and the organic fraction was dried (Na_2SO_4). Removal of the solvent *in vacuo* gave

a residue which was purified by silica gel column chromatography using ethyl acetate-hexane (2:3, v/v) as eluent to yield **(98)** as a yellow oil (1.63 g, 97%).

^{1}H NMR ($CDCl_3$): δ 7.32 (d, J = 7.8 Hz, 1H, aryl H-4), 7.26 (d, J = 7.8 Hz, 1H, aryl H-6), 7.07 (t, J = 7.8 Hz, aryl H-5), 5.71 - 5.77 (m, 3H, $COOCH_2OCO$ and N*H*), 5.42 (s, 1H, H-4), 4.96 (septet, J = 6.3 Hz, 1H, C*H*Me_2), 2.31 - 2.36 (m, 7H, C-2 and C-6 CH_3 and C*H*Pr^n_2), 1.18 - 1.59 (m, 11H, $CHCH_3$ and two $CH_2CH_2CH_3$), 1.02 (d, J = 6.3 Hz, 3H, $CHCH_3$), 0.85 (t, J = 7.2 Hz, 6H, two $CH_2CH_2CH_3$).

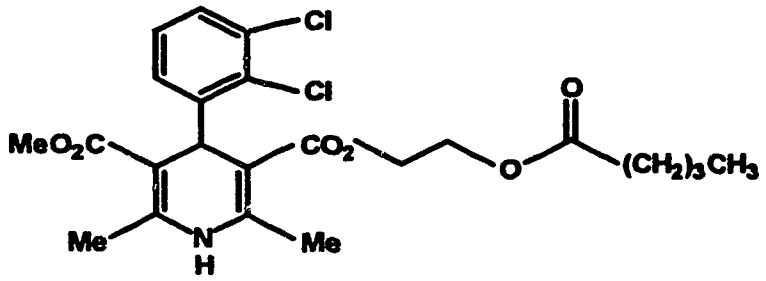
IR (film): 3321 (NH), 1732 (C=O) cm^{-1}.

Anal. Calcd. for $C_{27}H_{35}Cl_2NO_6.1/4H_2O$: C, 59.51; H, 6.57; N, 2.57.

Found: C, 59.35; H, 6.75; N, 2.51.

4.4.6.0.0.0. Syntheses of 3-(2-pentanoyloxyethyl) 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylates (108 - 109)

4.4.6.1.0.0. Syntheses of 3-(2-pentanoyloxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (108) (Procedure G)



(108)

Valeryl chloride (0.497 g, 4.12 mmol) was added to a solution of **(102)** (1.50 g, 3.75 mmol) and triethylamine (0.78 ml, 5.63 mmol) in dry CH_2Cl_2 (30 ml) at 0°C. The reaction was then allowed to proceed for 16 hours at 25°C. This solution was successively washed with HCl (0.5 N, 3 x 15 ml), $NaHCO_3$ (10% w/v, 3 x 15 ml) and water (3 x 15 ml), and the organic fraction was dried (Na_2SO_4). Removal of the solvent *in vacuo* gave a residue that was purified by silica gel column chromatography using ethyl acetate-hexane (1:2, v/v) as eluent to afford a yellow oil which on crystallization

from ethyl acetate-hexane afforded (**108**) as a white crystalline solid (1.84 g, 98%); mp 85 - 86°C.

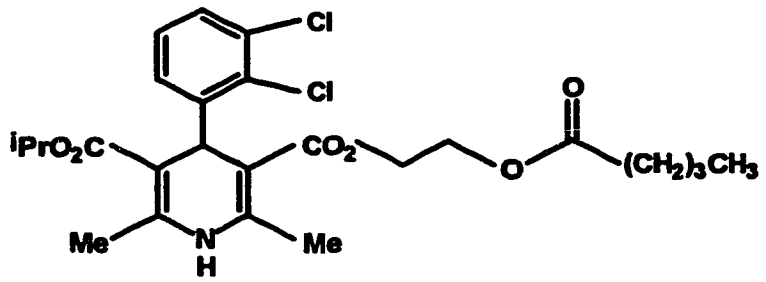
^{1}H NMR ($CDCl_3$): δ 7.31 (dd, J = 7.7 Hz, J = 1.6 Hz, 1H, aryl H-4), 7.25 (dd, J = 7.7 Hz, J = 1.6 Hz, 1H, aryl H-6), 7.07 (t, J = 7.7 Hz, 1H, aryl H-5), 5.69 (br s, 1H, N*H*), 5.46 (s, 1H, H-4), 4.23 (s, 4H, COOC*H*$_2$C*H*$_2$OCO), 3.62 (s, 3H, OC*H*$_3$), 2.23 - 2.38 (m, 8H, C-2 and C-6 C*H*$_3$ and OCOC*H*$_2$CH$_2$CH$_2$CH$_3$), 1.58 (pentet, J = 7.3 Hz, 2H, CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.35 (hexet, J = 7.3 Hz, 2H, CH$_2$CH$_2$C*H*$_2$CH$_3$), 0.91 (t, J = 7.3 Hz, 3H, CH$_2$CH$_2$CH$_2$C*H*$_3$).

IR (KBr): 3334 (NH), 1719, 1700, 1647 (C=O) cm^{-1}.

Anal. Calcd. for $C_{23}H_{27}Cl_2NO_6$.: C, 57.03; H, 5.62; N, 2.89.

Found: C, 56.98; H, 5.62; N, 2.88.

4.4.6.2.0.0. Syntheses of 3-(2-pentanoyloxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (109)



(109)

The preparation of (**109**) was similar to the method described in Procedure G which involved the reaction of (**103**) (1.50 g, 3.5 mmol), valeryl chloride (0.465 g, 3.85 mmol) and triethylamine (0.73 ml, 5.25 mmol). Purification of the product by elution from a silica gel column using ethyl acetate-hexane (1:2, v/v) and then recrystallization of the product from isopropyl ether-hexane afforded (**109**) as a white crystalline solid (1.70 g, 95%); mp 83 - 85°C.

^{1}H NMR ($CDCl_3$): δ 7.31 (dd, J = 7.9 Hz, J = 1.6 Hz, 1H, aryl H-4), 7.25 (dd, J = 7.9 Hz, J = 1.6 Hz, 1H, aryl H-6). 7.06 (t, J = 7.9 Hz, 1H, aryl H-5), 5.63 (br s, 1H, N*H*), 5.42 (s, 1H, H-4), 4.96 (septet, J = 6.3 Hz, 1H, C*H*Me$_2$), 4.23 (s, 4H,

COOCH_2CH_2CCO), 2.25 - 2.31 (m, 8H, C-2 and C-6 CH_3 and OCOCH_2CH_2CH_2CH_3), 1.58 (pentet, J = 7.4 Hz, 2H, CH_2CH_2CH_2CH_3), 1.32 (hextet, J = 7.4 Hz, 2H, $CH_2CH_2CH_2$CH_3), 1.24 (d, J = 6.3 Hz, 3H, CHCH_3), 1.03 (d, J = 6.3 Hz, 3H, CHCH_3), 0.90 (t, J = 7.4 Hz, 3H, $CH_2CH_2CH_2$CH_3).

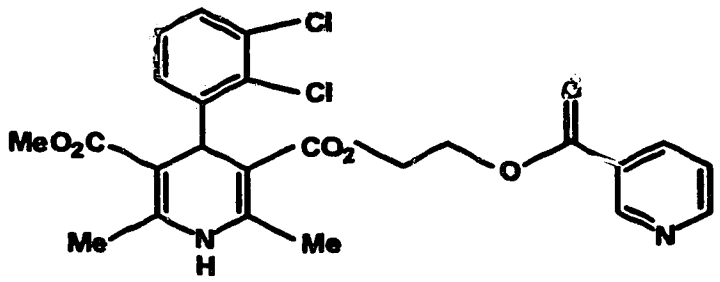
IR (KBr): 3349 (NH), 1728, 1696, 1649 (C=O) cm^{-1}.

Anal. Calcd. for $C_{25}H_{31}Cl_2NO_6$: C, 58.60; H, 6.10; N, 2.73.

Found: C, 58.67; H, 6.04; N, 2.71.

4.4.7.0.0.0. Syntheses of 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-alkyl 1,4-dihydro-2,6-dimethyl-4-(substituted-phenyl)-3,5-pyridinedicarboxylates (118 - 120)

4.4.7.1.0.0. Synthesis of 3-[2-(3-pyridylcarbonyloxy)ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (112)



(112)

The title compound (**112**) was prepared according to Procedure G by condensation of (**102**) (4.53 g, 11.3 mmol), triethylamine (3.7 g, 37 mmol) and nicotinoyl chloride hydrochloride (3.27 g, 18.4 mmol). Upon completion of the reaction, the reaction mixture was washed with water (3 x 25 ml) and the organic fraction was dried (Na_2SO_4). Removal of the solvent *in vacuo* yielded a yellow foam which was recrystallized from ethyl acetate-hexane to afford (**112**) as a light yellow crystalline solid (5.14 g, 90%); mp 146 - 148°C.

^{1}H NMR ($CDCl_3$): δ 8.96 (d, J = 1.4 Hz, 1H, pyridyl H-2), 8.86 (d, J = 5.0 Hz, 1H, pyridyl H-6), 8.61 (dd, J = 7.9 Hz, J = 1.4 Hz, 1H, pyridyl H-4), 7.77 (dd, J = 7.9 Hz, J = 5.0 Hz, 1H, pyridyl H-5), 7.36 (dd, J = 7.9 Hz, J = 1.6 Hz, 1H, aryl H-4). 7.04 - 7.10

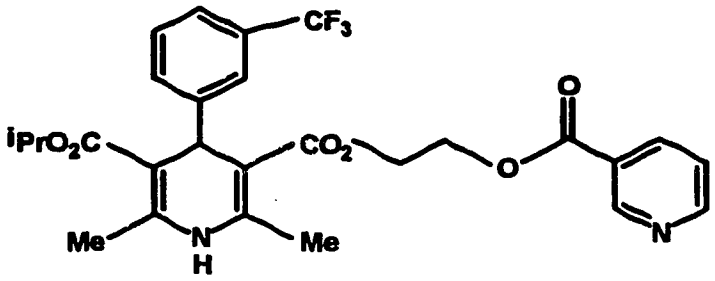
(m, 2H, aryl H-5 and H-6), 5.93 (s, 1H, N*H*), 5.46 (s, 1H, H-4), 4.52 - 4.61 and 4.27 - 4.31 (two m, 4H total, COOC*H*$_2$C*H*$_2$OCO), 3.60 (s, 3H, COOC*H*$_3$), 2.39 and 2.33 (two s, 3H each, C-2 and C-6 C*H*$_3$).

IR (KBr): 3345 (NH), 1713 (C=O), 736 (CH pyridine) cm^{-1}.

Anal. Calcd. for $C_{24}H_{22}Cl_2N_2O_6.1/2H_2O$: C, 56.04; H, 4.51; N, 5.45.

Found: C, 56.44; H, 4.33; N, 5.49.

4.4.7.1.1.0. Synthesis of 3-[2-(3-pyridylcarbonyloxy)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-trifluoromethylphenyl)-3,5-pyridinedicarboxylate (113)



(113)

Compound (**113**) was prepared according to Procedure G by reaction of (**110**) (3.30 g, 7.73 mmol), triethylamine (3.2 ml, 2.3 mmol) and nicotinoyl chloride hydrochloride (1.51 g, 8.5 mmol). Upon completion of the reaction, the reaction mixture was washed with water (3 x 25 ml) and the organic fraction was dried (Na_2SO_4). Evaporation of the solvent *in vacuo* yielded a yellow foam which was recrystallized from ether-hexane to afford (**113**) as a pale yellow crystalline solid (3.50 g, 85%); mp 122 - 124°C.

^{1}H NMR ($CDCl_3$): δ 9.16 (dd, J = 2.0 Hz, J = 0.8 Hz, 1H, pyridyl H-2), 8.80 (dd, J = 5.0 Hz, J = 2.0 Hz, 1H, pyridyl H-6). 8.22 (ddd, J = 7.9 Hz, J = 2.0 Hz, J = 2.0 Hz, 1H, pyridyl H-4), 7.52 (s, 1H, aryl H-2), 7.46 (d, J = 7.9 Hz, 1H, aryl H-4), 7.37 (dd, J = 7.9 Hz, J = 5.0 Hz, 1H, pyridyl H-5), 7.31 (d, J = 7.9 Hz, 1H, aryl H-6), 7.24 (t, J = 7.9 Hz, 1H, aryl H-5), 5.66 (s, 1H, N*H*), 5.02 (s, 1H, H-4), 4.93 (septet, J = 6.2 Hz, 1H,

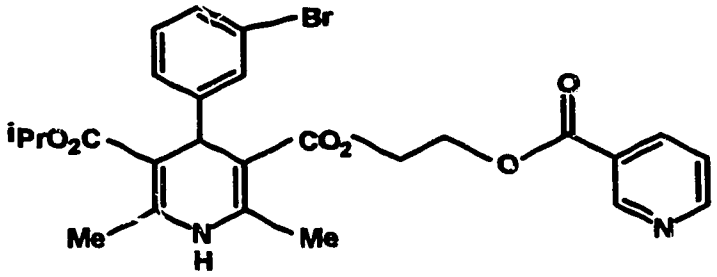
C*H*Me$_2$), 4.31 - 4.53 (m, 4H, COOC*H*$_2$C*H*$_2$OCO), 2.37, 2.35 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.20 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.05 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3355 (NH), 1731, 1697 (C=O), 745 (CH pyridine) cm^{-1}.

Anal. Calcd. for $C_{27}H_{27}F_3N_2O_6$: C, 60.90; H, 5.11; N, 5.26.

Found: C, 60.76; H, 4.86; N, 5.05.

4.4.7.1.2.0. Synthesis of 3-[2-(3-pyridylcarbonyloxy)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-bromophenyl)-3,5-pyridinedicarboxylate (114)



(114)

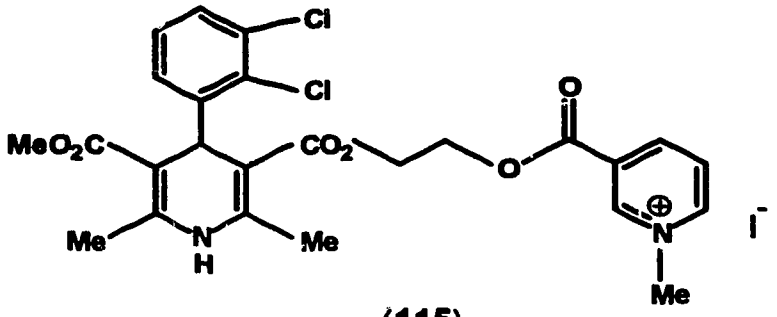
Compound (114) was prepared using the method described in Procedure G by reaction of (111) (5.27 g, 12.0 mmol), triethylamine (5 ml, 36 mmol) and nicotinoyl chloride hydrochloride (3.21 g, 18 mmol). Upon completion of the reaction, the reaction mixture was washed with water (3 x 25 ml) and the organic layer was dried (Na_2SO_4). Removal of the solvent *in vacuo* yielded a yellow foam which was recrystallized from CH_2Cl_2-hexane to afford (114) as a light yellow crystalline solid (6.20 g, 95%); mp 145 - 146°C.

^{1}H NMR ($CDCl_3$): δ 9.17 (d, J = 1.9 Hz, 1H, pyridyl H-2), 8.80 (dd, J = 4.8 Hz, J = 1.9 Hz, 1H, pyridyl H-6), 8.23 (ddd, J = 8 Hz, J = 1.9 Hz, J = 1.9 Hz, 1H, pyridyl H-4), 7.38 - 7.42 (m, 2H, pyridyl H-5 and aryl H-2), 7.13 - 7.20 (m, 2H, aryl H-4 and H-6), 6.97 (t, J = 7.8 Hz, 1H, aryl H-5), 5.65 (s, 1H, N*H*), 4.90 - 4.98 (m, 2H, C-4 and C*H*Me$_2$), 4.46 - 4.53 and 4.31 - 4.38 (two m, 4H total, COOC*H*$_2$C*H*$_2$OCO), 2.36 and 2.33 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.21 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.09 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3342, 3204 (NH), 1729, 1693 (C=O), 748 (CH, pyridine) cm^{-1}.

Anal. Calcd. for $C_{26}H_{27}BrN_2O_6.1/2H_2O$: C, 56.53; H, 5.11; N, 5.07.
Found: C, 56.77; H, 4.71; N, 5.14.

4.4.7.2.0.0. Synthesis of 3-[2-(1-methylpyridinium-3-carbonyloxy)ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate iodide (115) (Procedure H)



(115)

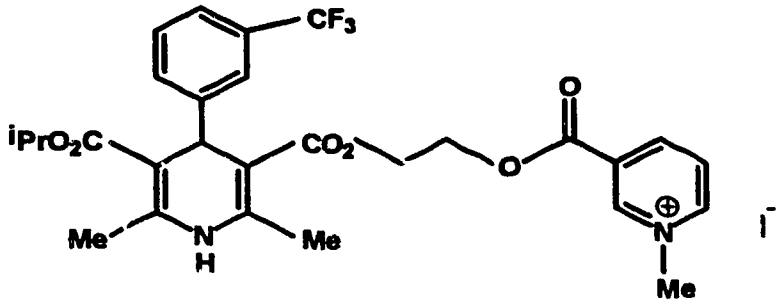
A solution of (**112**) (0.86 g, 1.70 mmol) and iodomethane (0.53 ml, 8.52 mmol) in dry acetone (50 ml) was refluxed for 16 hours. The yellow ppt which formed was filtered and washed with dry ether (3 x 15 ml). This light yellow solid product was dried *in vacuo* to afford (**115**) (1.00g, 90.8%); mp 205 - 207°C.

^{1}H NMR (DMSO-d_6): δ 9.46 (s, 1H, N*H*), 9.20 (d, J = 6.4 Hz, 1H, pyridinium H-6), 9.01 (s, 1H, pyridinium H-2), 8.73 (d, J = 8.2 Hz, 1H, pyridinium H-4), 8.27 (dd, J = 8.2 Hz, J = 6.4 Hz, 1H, pyridinium H-5), 7.22 - 7.29 (m, 2H, aryl H-4 and H-6), 7.16 (t, J = 7.7 Hz, 1H, aryl H-5), 5.30 (s, 1H, H-4), 4.51 (br m, 2H, COOC*H*$_2$CH$_2$OCO), 4.24 - 4.43 (m, 5H, NC*H*$_3$ and COOCH$_2$C*H*$_2$OCO), 3.45 (s, 3H, COOC*H*$_3$), 2.26 amd 2.21 (two s, 3H each, C-2 and C-6 C*H*$_3$).

IR (KBr): 3443 (NH), 1740, 1698, 1641 (C=O) cm^{-1}.

Anal. Calcd. for $C_{25}H_{25}Cl_2IN_2O_6$: C, 46.39; H, 3.98; N, 4.33.
Found: C, 46.11; H,3.76; N, 4.20.

4.4.7.2.1.0. Synthesis of 3-[2-(1-methylpyridinium-3-carbonyloxy)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-trifluoromethylphenyl)-3,5-pyridine-dicarboxylate iodide (116)



(116)

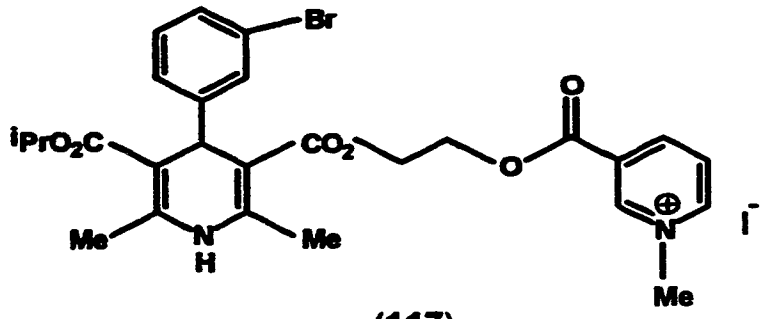
The title compound (**116**) was prepared according to the method described in Procedure H by reaction of (**113**) (0.43 g, 0.81 mmol), iodomethane (0.25 ml, 4.02 mmol). No solid precipitate was formed in this reaction. The product (**116**) was isolated as a yellow foam after removal of the solvent *in vacuo* (0.52g, 96%).

^{1}H NMR ($CDCl_3$): δ 9.70 (d, J = 6.1 Hz, 1H, pyridinium H-6), 9.28 (s, 1H, pyridinium H-2), 8.76 (d, J = 7.7 Hz, 1H, pyridinium H-4), 8.27 (dd, J = 7.7 Hz, J = 6.1 Hz, 1H, pyridinium H-5), 7.43 - 7.47 (m, 2H, aryl H-2 and H-4), 7.27 - 7.30 (m, 2H, aryl H-5 and H-6), 6.80 (s, 1H N*H*), 4.88 - 4.97 (m, 2H, H-4 and C*H*Me_2), 4.73 (s, 3H, NCH_3), 4.48 - 4.65 and 4.33 - 4.41 (two m, 4H total, COOCH_2CH_2OCO), 2.38 and 2.35 (two s, 3H each, C-2 and C-6 CH_3), 1.22 (d, J = 6.0 Hz, 3H, CHCH_3), 1.09 (d, J = 6.0 Hz, 3H, CHCH_3).

IR (KBr): 3422 (NH), 1743, 1685 (C=O) cm^{-1}.

Compound (**116**) was used immediately to synthesize (**119**).

4.4.7.2.2.0. Synthesis of 3-[2-(1-methylpyridinium-3-carbonyloxy)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-bromophenyl)-3,5-pyridinedicarboxylate iodide (117)



(117)

Thw title compound (117) was prepared according to the method described in Procedure H by reaction of (114) (3.83 g, 7.05 mmol) with iodomethane (2.2 ml, 3.52 mmol). The product, which did not precipitate from the reaction mixture, was isolated as a yellow foam after evaporating the solvent to afford (117) (4.59 g, 95%).

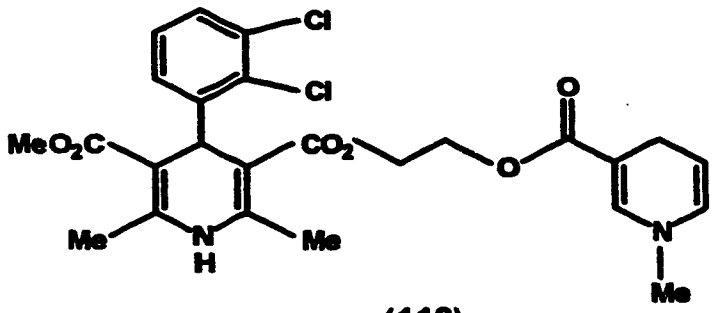
^{1}H NMR ($CDCl_3$): δ 9.75 (d, J = 6.0 Hz, 1H, pyridinium H-6), 9.16 (s, 1H, pyridinium H-2), 8.73 (d, J = 8.1 Hz, 1H, pyridinium H-4), 8.27 (dd, J = 8.1 Hz, J = 6.0 Hz, 1H, pyridinium H-5), 7.31 (d, J = 1.7 Hz, 1H, aryl H-2), 7.18 (d, J = 7.5 Hz, 1H, aryl H-4), 7.08 (dd, J = 7.5 Hz, J = 1.7 Hz, 1H, aryl H-6), 7.01 (t, J = 7.5 Hz, 1H, aryl H-5), 6.73 (s, 1H, N*H*), 4.95 (septet, J = 6.3 Hz, 1H, C*H*Me$_2$), 4.89 (s, 1H, H-4), 4.73 (s, 3H, NC*H*$_3$), 4.49 - 4.64 and 4.28 - 4.34 (two m, 4H total, COOC*H*$_2$C*H*$_2$OCO), 2.40 and 2.35 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.23 (d, J = 6.3 Hz, 3H, CHC*H*$_3$), 1.14 (d, J = 6.3 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3235, 3081 (NH), 1737, 1690 (C=O) cm^{-1}.

Anal. Calcd. for $C_{27}H_{30}BrIN_2O_6.1/2H_2O$: C, 46.70; H, 4.50; N, 4.03.

Found: C, 46.80; H, 4.20; N, 3.98.

4.4.7.3.0.0. Synthesis of 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (118) (Procedure I)



(118)

Diethyl ether (10 ml) was added to a solution of (**115**) (0.27 g, 0.421 mmol) and $NaHCO_3$ (0.18 g, 2.1 mmol) in degassed water (10 ml) under an argon atmosphere with stirring. Sodium dithionite (0.37 g, 2.1 mmol) was then added. The reaction was allowed to proceed with stirring at 25°C for 1 hour after which the organic phase was separated and washed with degassed water (3 x 10 ml). The organic phase was dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a yellow solid which on recrystallization from MeOH yielded (**118**) as a yellow crystalline solid (0.126 g, 57.5%); mp 160 - 165°C.

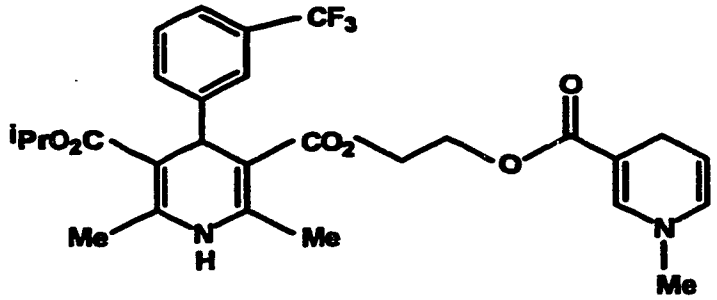
^{1}H NMR (DMSO-d_6): δ 8.98 (s, 1H, N*H*), 7.36 (dd, J = 7.7 Hz, J = 1.7 Hz, 1H, aryl H-4), 7.28 (dd, J = 7.7 Hz, J = 1.7 Hz, 1H, aryl H-6), 7.21 (t, J = 7.7 Hz, 1H, aryl H-5), 6.87 (s, 1H, dihydropyridyl H-2), 5.83 (dd, J = 7.9 Hz, J = 1.4 Hz, 1H, dihydropyridyl H-6), 5.31 (s, 1H, H-4), 4.67 - 4.72 (m, 1H, dihydropyridyl H-5), 4.09 - 4.17 (br m, 4H, COOC*H*$_2$C*H*$_2$OCO), 3.48 (s, 3H, COOC*H*$_3$), 2.91 (s, 3H, NC*H*$_3$), 2.87 (br s, 2H, dihydropyridyl H-4), 2.25 and 2.22 (two s, 3H each, C-2 and C-6 C*H*$_3$).

IR (KBr): 3329 (NH), 1701, 1658 (C=O) cm^{-1}.

Anal. Calcd. for $C_{25}H_{26}Cl_2N_2O_6.1/2H_2O$: C, 56.61; H, 5.13; N, 5.28.

Found: C, 56.65; H, 5.25; N, 4.91.

4.4.7.3.1.0. Synthesis of 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-trifluoromethylphenyl)-3,5-pyridine-dicarboxylate (119)



(119)

The title compound **(119)** was synthesized using the method described in Procedure I by reduction of **(116)** (0.53 g, 0.786 mmol), with sodium dithionite (0.68 g, 3.93 mmol) in the presence of $NaHCO_3$ (0.33 g, 3.93 mmol). The product **(119)**, which was not recrystallized, was isolated as a yellow foam (0.358 g, 83%).

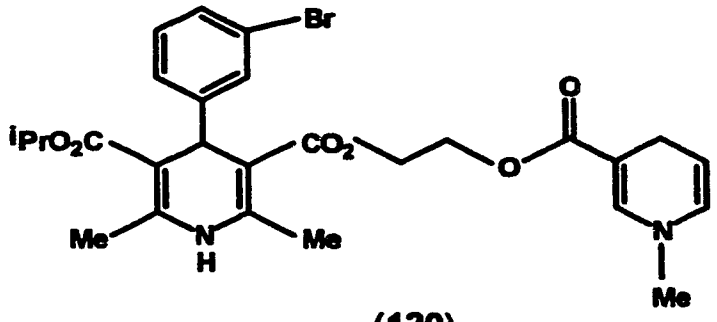
^{1}H NMR (DMSO-d_6): δ 8.95 (s, 1H, N*H*), 7.42 - 7.52 (m, 4H, aryl-H), 6.96 (s, 1H, dihydropyridyl H-2), 5.83 (d, J = 6.6 Hz, 1H, dihydropyridyl H-6), 4.87 (s, 1H, H-4), 4.79 (septet, J = 6.3 Hz, 1H, C*H*Me$_2$), 4.67 - 4.71 (m, 1H, dihydropyridyl H-5), 4.05 - 4.21 (m, 4H, COOCH_2CH_2OCO), 2.90 (s, 5H, NCH_3 and dihydropyridyl H-4), 2.26 (s, 6H, C-2 and C-6 CH_3), 1.17 (d, J = 6.3 Hz, 3H, CHCH_3), 0.99 (d, J = 6.3 Hz, 3H, CHCH_3).

IR (KBr): 3348 (NH), 1687, 1601 (C=O) cm^{-1}.

Anal. Calcd. for $C_{28}H_{31}F_3N_2O_6$: C, 61.31; H, 5.70; N, 5.11.

Found: C, 60.93; H, 5.46; N, 4.89.

4.4.7.3.2.0. Synthesis of 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-bromophenyl)-3,5-pyridinedicarboxylate (120)



(120)

The title compound (**120**) was synthesized using the method described in Procedure I by reduction of (**117**) (0.15 g, 0.22 mmol) with sodium dithionite (0.19 g, 1.1 mmol) in the presence of $NaHCO_3$ (0.09 g, 1.1 mmol). The product (**120**), which was not recrystallized, was isolated as a yellow foam (0.13 g, 83%).

^{1}H NMR (DMSO-d_6): δ 8.90 (s, 1H, N*H*), 7.28 - 7.30 (m, 2H, aryl H-2 and H-4), 7.11 - 7.19 (m, 2H, aryl H-6 and H-5), 6.97 (d, J = 1.4 Hz, 1H, dihydropyridyl H-2), 5.84 (dd, J = 7.9 Hz, J = 1.4 Hz, 1H, dihydropyridyl H-6), 4.76 - 4.84 (m, 2H, C*H*Me$_2$ and H-4), 4.68 - 4.73 (m, 1H, dihydropyridyl H-5), 4.09 - 4.18 (m, 4H, COOCH_2CH_2OCO), 2.94 (m, 2H, dihydropyridyl H-4), 2.91 (s, 3H, NCH_3), 2.26 and 2.25 (two s, 3H each, C-2 and C-6 CH_3), 1.17 (d, J = 6.2 Hz, 3H, CHCH_3), 1.04 (d, J = 6.2 Hz, 3H, CHCH_3).

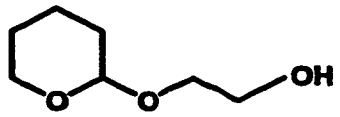
IR (KBr): 2976 (NH), 1695 (C=O) cm^{-1}.

Anal. Calcd. for $C_{27}H_{31}BrN_2O_6.1/2H_2O$: C, 57.05; H, 5.67; N, 4.93.

Found: C, 57.43; H, 5.68; N, 5.11.

4.4.8.0.0.0. Synthesis of 3-ethyl 5-methyl 1,4-dihydro-2-[2-[(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethoxy]methyl]-6-methyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (131)

4.4.8.1.0.0. Synthesis of 2-tetrahydropyranyl 2-hydroxyethyl ether (124)



(124)

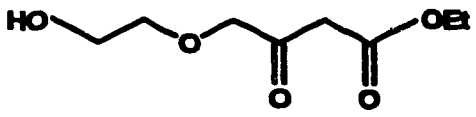
This method for monoprotection of ethylene glycol was based on the method described by Boom and Herschied.[227] A solution of 3,4-dihydro-*2H*-pyran (16.82 g, 200 mmol) in dry THF (50 ml) was added to a solution of dry ethylene glycol (31.0 g, 500 mmol) in dry THF (50 ml) containing *p*-toluenesulphonic acid (1.0 g, 5.26 mmol) and the reaction was allowed to proceed at 25°C for 16 hours with stirring. The reaction mixture was made alkaline by the addition of a saturated ammonia solution in MeOH until the pH was 10. After removing the solvent *in vacuo,* the residue was dissolved in CH_2Cl_2 (50 ml), washed with saturated brine solution (3 x 15 ml) and the organic phase was dried (Na_2SO_4). Removal of the solvent *in vacuo* and purification of the product obtained by elution from a silica gel column using ethyl acetate-hexane (1:1, v/v) as eluent afforded **(124)** as a light yellow liquid (15.96 g, 54.6%). The first component eluted from the column was the *bis*-(2-tetrahydropyranyl)ethyl ether (1.3 g, 2.8%) that was discarded.

^{1}H NMR ($CDCl_3$): δ 4.50 (m, 1H, pyranyl H-2), 3.8 - 4.0 and 3.4 - 3.5 (two m, 1H each, pyranyl H-6), 3.5 - 3.8 (m, 4H, HOCH_2CH_2O), 3.05 - 3.24 (br s, 1H, OH), 1.60 - 2.0 (m, 2H, pyranyl H-3), 1.34 - 1.60 (m, 4H, pyranyl H-4 and H-5).

IR (film): 3080 - 3720 (br) (OH) cm^{-1}.

The product **(124)** was used in the subsequent reaction.

4.4.8.2.0.0. Synthesis of ethyl 4-(2-hydroxyethoxy)acetoacetate (127)



(127)

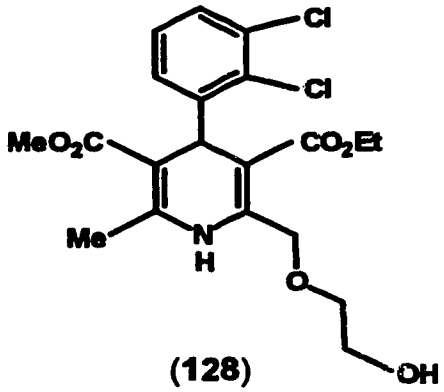
A solution of **(124)** (15.96 g, 109.2 mmol) in dry THF (10 ml) was added to a stirred suspension of NaH (8.43 g, 80% dispersion in oil, 275 mmol) in dry THF (20 ml) under an argon atmosphere. After stirring at 25°C for 1 hour, this mixture was cooled to 5°C and a solution of ethyl 4-chloroacetoacetate **(125)** (18.92 g, 110 mmol) in dry THF (10 ml) was added slowly. The reaction was allowed to proceed at 25°C for 16 hours. Ethanol (5 ml) was added to the reaction mixture and the pH was adjusted to 6 - 7 using 0.5 N HCl. The precipitate of NaCl, which was formed upon acidification, was dissolved by the addition of a minimal amount of water. After separating the aqueous layer, the solvent from the organic phase was removed *in vacuo* and the residue obtained was treated with a solution of EtOH:HCl (0.1N) (100:1, v/v) (20 ml). This mixture was then refluxed for 10 min before adding water (10 ml) at 25°C. The solvent with the water was removed as much as possible by evaporation *in vacuo*. The residue was then taken up in ethyl acetoacetate (50 ml) and dried (Na_2SO_4). Purification of the product by elution from a silica gel column using ethyl acetate-hexane (1:1, v/v) as eluent afforded **(127)** as a yellow liquid (13.5 g, 65%).

^{1}H NMR ($CDCl_3$): δ 4.95 (s, 1H, OH), 4.14 - 4.23 (m, 3H, C*H*$_2$CH$_3$ and HOCH$_2$CH*H'*O), 3.49 - 3.73 (m, 4H, HOC*H*$_2$*CH*H'*O* and OCH*H'*CO), 3.43 (d, J_{gem} = 11.3 Hz, 1H, OC*H*H'CO), 2.63 (d, J_{gem} = 15.3 Hz, 1H, COCH*H'*CO), 2.47 (d, J_{gem} = 15.3 Hz, 1H, COC*H*H'CO), 1.26 (t, J = 7.2 Hz, 3H, CH$_2$C*H*$_3$).

IR (film): 3072 - 3747 (br) (OH), 1737 (C=O) cm^{-1}.

The product **(127)** was used in the subsequent reaction.

4.4.8.3.0.0. Synthesis of 3-ethyl 5-methyl 1,4-dihydro-2-[(2-hydroxy-ethoxy)methyl]-6-methyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (128)



(128)

A solution of **(127)** (4.25 g, 22.3 mmol), methyl 2-(2,3-dichlorobenzylidene)acetoacetate (*E*)- and (*Z*)-**(87)** (5.32 g, 22.3 mmol) and ammonium acetate (1.92 g, 25 mmol) in EtOH (100 ml) was heated at reflux for 16 hours. After removing the solvent *in vacuo*, the residue was dissolved in CH_2Cl_2 (50 ml) and washed with water (3 x 25 ml). Removal of the solvent from the organic phase *in vacuo* and purification of the residue obtained by silica gel column chromatography with ethyl acetate-hexane (1:1, v/v) as eluent afforded a yellow solid which on recrystallization from CH_2Cl_2-hexane yielded **(128)** as a light yellow solid (2.47 g, 32%); mp 129 - 131°C.

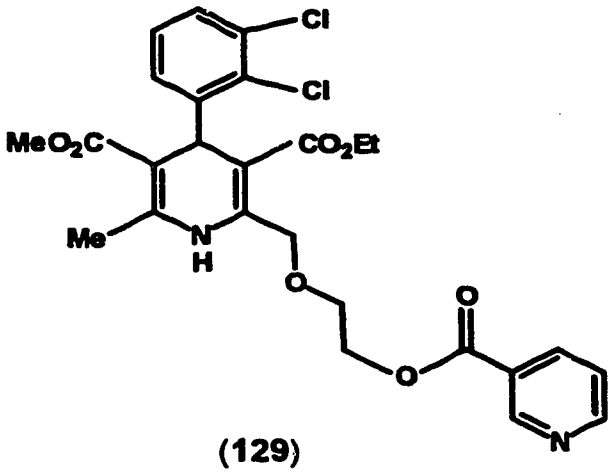
^{1}H NMR ($CDCl_3$): δ 7.45 (s, 1H, N*H*), 7.31 (dd, J = 7.7 Hz, J = 1.6 Hz, 1H, aryl H-4), 7.24 - 7.27 (m, 1H, aryl H-6), 7.08 (t, J = 7.7 Hz, 1H, aryl H-5), 5.48 (s, 1H, H-4), 4.82 (d, J_{gem} = 16.7 Hz, 1H, C-2 CH*H'*O), 4.73 (d, J_{gem} = 16.7 Hz, 1H, C-2 C*H*H'O), 4.05 (q, J = 7.1 Hz, 2H, COOC*H*$_2$CH$_3$), 3.88 (t, J = 4.3 Hz, 2H, OC*H*$_2$CH$_2$OH), 3.70 (m, 2H, C*H*$_2$OH), 3.62 (s, 3H, COOC*H*$_3$), 2.35 (s, 3H, C-6 C*H*$_3$), 1.84 (br s, 1H, OH, exchanges with D_2O), 1.18 (t, J = 7.1 Hz, 3H, CH$_2$C*H*$_3$).

IR (KBr): 3309 - 3557 (br) (OH), 3453 (NH), 1590, 1644 (C=O) cm^{-1}.

Anal. Calcd. for $C_{20}H_{23}Cl_2NO_6$: C, 54.07; H, 5.22; N, 3.15.

Found: C, 53.99; H, 5.19; N, 3.13.

4.4.8.4.0.0. Synthesis of 3-ethyl 5-methyl 1,4-dihydro-2-[2-[(3-pyridylcarbonyloxy)ethoxy]methyl]-6-methyl-4-(2,3-dichlorophenyl)-3,5-pyridine-dicarboxylate (129)



(129)

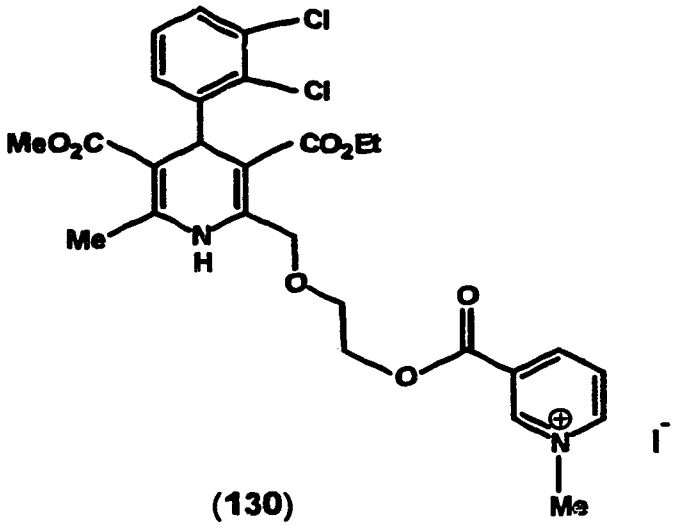
The method outlined in procedure G was employed for the synthesis of **(129)** which involved the reaction of **(128)** (2.21 g, 4.97 mmol), nicotinoyl chloride hydrochloride (0.89 g, 5 mmol) and triethylamine (1.4 ml, 10 mmol). The reaction mixture was washed with water (3 x 15 ml), the solvent from the organic phase was removed after drying (Na_2SO_4) and the residue obtained was recrystallized from ethyl acetate-hexane to yield **(129)** as a light yellow crystalline solid (5.14 g, 90%); mp 146 - 148°C.

^{1}H NMR ($CDCl_3$): δ 9.27 (d, J = 1.2 Hz, 1H, pyridyl H-2), 8.81 (dd, J = 4.8 Hz, J = 1.2 Hz, 1H, pyridyl H-6), 8.34 (ddd, J = 7.8 Hz, J = 1.2 Hz, J = 1.2 Hz, 1H, pyridyl H-4), 7.43 (dd, J = 7.8 Hz, J = 4.8 Hz, 1H, pyridyl H-5), 7.22 - 7.29 (m, 2H, aryl H-4 and H-6), 7.15 (s, 1H, N*H*), 7.05 (t, J = 7.8 Hz, 1H, aryl H-5), 5.45 (s, 1H, H-4), 4.83 (d, J_{gem} = 15.9 Hz, 1H, C-2 CH*H'*O), 4.74 (d, J_{gem} = 15.9 Hz, 1H, C-2 C*H*H'O), 4.58 - 4.69 (m, 2H, OCH_2CH_2OCO), 4.04 (q, J = 7.1 Hz, 2H, $COOCH_2CH_3$), 3.93 (t, J = 4.5 Hz, 2H, OCH_2CH_2OCO), 3.60 (s, 3H, $COOCH_3$), 2.30 (s, 3H, C-6 CH_3), 1.16 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$).

IR (KBr): 3386 (NH), 1726, 1691 (CO), 743 (CH pyridine) cm^{-1}.

This product **(129)** was used as an intermediate for the synthesis of **(130)**.

4.4.8.5.0.0. Synthesis of 3-ethyl 5-methyl 1,4-dihydro-2-[2-(1-methylpyridinium-3-carbonyloxyethoxy)methyl]-6-methyl-4-(2,3-dichlorophenyl)-3,5-pyridine-dicarboxylate iodide (130)



(130)

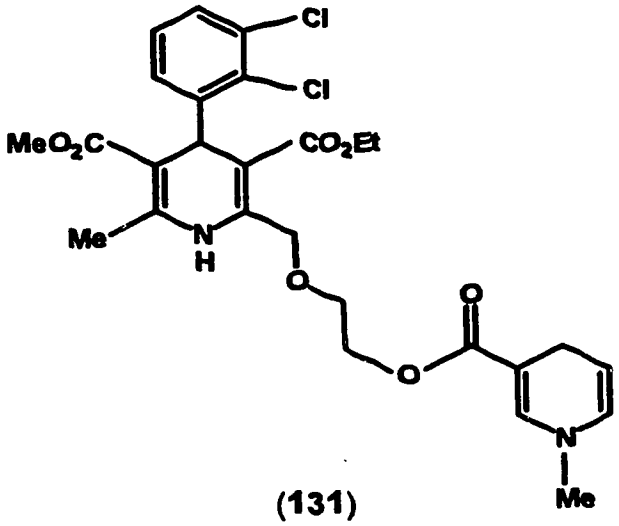
Procedure H was employed for the synthesis of **(130)**. Thus reaction of **(129)** (4.2 g, 7.66 mmol) with iodomethane (2.38 ml, 38.3 mmol) afforded **(130)** as a yellow foam (4.5 g, 85%).

^{1}H NMR ($CDCl_3$): δ 9.70 (d, J = 6.1 Hz, 1H, pyridinium H-6), 9.63 (s, 1H, pyridinium H-2), 9.00 (d, J = 8.1 Hz, 1H, pyridinium H-4), 8.29 (dd, J = 8.1 Hz, J = 6.1 Hz, pyridinium H-5), 7.31 (dd, J = 7.9 Hz, J = 1.6 Hz, 1H, aryl H-4), 7.25 (dd, J = 7.9 Hz, J = 1.6 Hz, 1H, aryl H-6), 7.08 (t, J = 7.9 Hz, 1H, aryl H-5), 5.45 (s, 1H, H-4), 4.93, (d, J_{gem} = 15.5 Hz, 1H, C-2, CH*H'*O), 4.79 - 4.84 (m, 4H, N^+CH_3, C-2, C*H*H'O), 4.70 - 4.72 (m, 2H, OCH_2CH_2OCO), 3.98 - 4.07 (m, 4H, OCH_2CH_2OCO, $COOCH_2CH_3$), 3.61 (s, 3H, $COOCH_3$), 2.37 (s, 3H, C-6 CH_3), 1.18 (t, J = 7.1 Hz, 3H, CH_2CH_3).

IR (KBr): 3400 (NH), 1734, 1690 (C=O) cm^{-1}.

Compound **(130)** was used immediately for the synthesis of **(131)**.

4.4.8.6.0.0. Synthesis of 3-ethyl 5-methyl 1,4-dihydro-2-[2-[(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethoxy]methyl]-6-methyl-4-(2,6-dichlorophenyl)-3,5-pyridinedicarboxylate (131)



(131)

Procedure I was employed for the synthesis of **(131)**. Thus reduction of **(130)** (2.36 g, 3.41 mmol) with sodium dithionite (2.97 g, 17 mmol) in the presence of $NaHCO_3$ (1.43 g, 17 mmol) yielded **(131)** as a yellow foam (1.83 g, 95 %).

^{1}H NMR (DMSO-d_6): δ 8.60 (s, 1H, N*H*), 7.40 (dd, J = 7.7 Hz, J = 1.7 Hz, 1H, aryl H-4), 7.30 (dd, J = 7.7 Hz, J = 1.7 Hz, 1H, aryl H-6), 7.24 (t, J = 7.7 Hz, 1H, aryl H-5), 7.06 (d, J = 1.4 Hz, 1H, dihydropyridyl H-2), 5.84 (dd, J = 7.9 Hz, J = 1.4 Hz, 1H, dihydropyridyl H-6), 5.36 (s, 1H, H-4), 4.57 - 4.73 (m, 3H, dihydropyridyl H-5 and C-2 $CH_2OCH_2CH_2$), 4.10 - 4.24 (m, 2H, OCH_2CH_2OCO), 3.97 (q, J = 7.1 Hz, 2H, $COOCH_2CH_3$), 3.67 (br t, J = 4.5 Hz, 2H, OCH_2CH_2OCO), 3.50 (s, 3H, $COOCH_3$), 2.95 (m, 2H, dihydropyridyl H-4), 2.92 (s, 3H, NCH_3), 2.28 (s, 3H, C-6 CH_3), 1.08 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$).

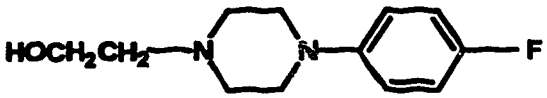
IR (KBr): 3507 (NH), 1696 (C=O) cm^{-1}.

Anal. Calcd. for $C_{27}H_{30}Cl_2N_2O_7.1/2H_2O$: C, 56.45; H, 5.44; N, 4.88.

Found: C, 56.16; H, 5.31; N, 4.77.

4.4.9.0.0.0. Synthesis of 3-[2-[4-(4-fluorophenyl)piperazinyl]ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (137)

4.4.9.1.0.0. Synthesis of 2-[4-(*p*-fluorophenyl)]piperazin-1-yl]ethanol (135)



(135)

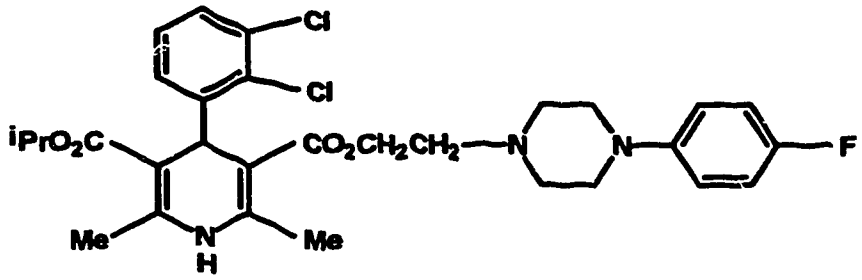
A solution of 1-(4-fluorophenyl)piperazine (4.5 g, 25 mmol), 2-bromoethanol (3.17 g, 25 mmol) and triethylamine (7 ml, 50.2 mmol) in acetone (50 ml) was refluxed for 24 hours. The solvent was removed *in vacuo* and the residue obtained was dissolved in CH_2Cl_2 (50 ml) and washed with water (3 x 25 ml). The organic phase was dried (Na_2SO_4), the solvent was removed, and the residue obtained was purified by silica gel column chromatography using CH_2Cl_2-MeOH (96:4, v/v) as eluent to give (**135**) as a white foam (3.08 g, 55%).

The assignment of the piperazinyl protons was based on the fact that the H-3 and H-5 protons are deshielded by the *p*-fluorophenyl substituent resulting in their appearance at lower field ($\delta = 3.15$), whereas the H-2 and H-6 piperazinyl protons which are not affected appear at higher field ($\delta = 2.71$).

^{1}H NMR ($CDCl_3$): δ 6.86 - 7.01 (m, 4H, aryl-H), 3.68 (t, J = 5.4 Hz, 2H, C*H*$_2$OH), 3.15 (t, J = 4.9 Hz, 4H, piperazinyl H-3 and H-5), 2.87 (s, 1H, OH, exchanges with D_2O), 2.71 (t, J = 4.9 Hz, 4H, piperazinyl H-2 and H-6), 2.64 (t, J = 5.4 Hz, 2H, CH_2C*H*$_2$N).

Product (**135**) was used immediately for the synthesis of (**136**) as described in section 4.4.1.2.0.0.

4.4.9.2.0.0. Synthesis of 3-[2-[4-(4-fluorophenyl)piperazinyl]ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (137)



(137)

Procedure C was used to synthesize (**137**). Thus, condensation of 2,3-dichlorobenzaldehyde (1.44 g, 8.2 mmol), 2-[4-(*p*-fluorophenyl)piperazin-1-yl]ethyl acetoacetate (**136**) (2.53 g, 8.2 mmol) and isopropyl 3-aminocrotonate (**74**) (1.18 g, 8.2 mmol) yielded the product which was purified by elution from a silica gel column using ethyl acetate-hexane as eluent (1:1, v/v) to give (**137**) as a white solid (2.33 g, 48%); mp 65 - 70°C.

^{1}H NMR ($CDCl_3$): δ 7.33 (dd, J = 7.9 Hz, J = 1.6 Hz, 1H, dichlorophenyl H-4), 7.25 (dd, J = 7.9 Hz, J = 1.6 Hz, 1H, dichlorophenyl H-6), 7.08 (t, J ≈ 7.9 Hz, 1H, dichlorophenyl H-5), 6.84 - 7.00 (m, 4H, fluorophenyl hydrogens), 5.64 (s, 1H, N*H*), 5.45 (s, 1H, H-4), 4.99 (septet, J = 6.3 Hz, 1H, C*H*Me$_2$), 4.10 - 4.29 (m, 2H, COOC*H*$_2$), 3.07 (br t, J = 4.6 Hz, 4H, piperazinyl H-3 and H-5), 2.50 - 2.64 (m, 6H, piperazinyl H-2, H-6 and CH_2CH_2N), 2.32 (s, 6H, C-2 and C-6 C*H*$_3$), 1.26 (d, J = 6.3 Hz, 3H, CHC*H*$_3$), 1.06 (d, J = 6.3 Hz, 3H, CHC*H*$_3$).

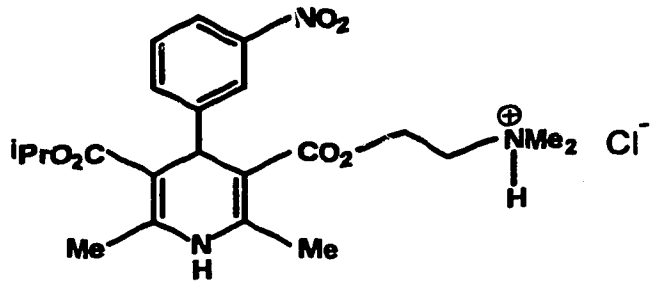
IR (KBr): 3472 (NH), 1700 (C=O) cm^{-1}.

Anal. Calcd. for $C_{30}H_{34}Cl_2FN_3O_4.1/2H_2O$: C, 60.10; H, 5.88; N, 7.01.

Found: C, 60.28; H, 5.71; N, 6.85.

4.4.10.0.0.0. Syntheses of 3-[2-(dimethylamino)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (139) and 3-[2-(trimethylammonium)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate iodide (140)

4.4.10.1.0.0. Synthesis of 3-[2-(dimethylamino)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate hydrochloride (139)



(139)

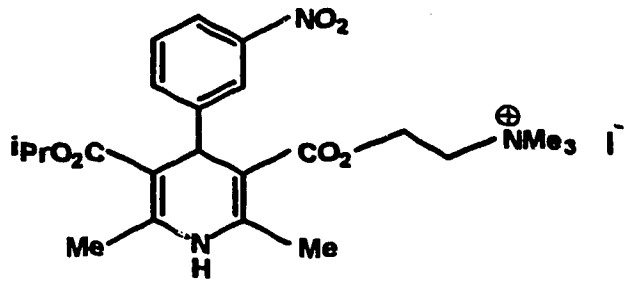
The title compound (**139**) was prepared according to Procedure C by condensation of 3-nitrobenzaldehyde (3.02 g, 20 mmol), 2-(N,N-dimethylamino)ethyl acetoacetate (**138**) (3.46 g, 20 mmol) and isopropyl 3-aminocrotonate (**74**) (2.86 g, 20 mmol). The compound (free base) was purified by elution from a silica gel column with CH_2Cl_2-MeOH (95:5, v/v) as eluent to afford a solid. A precooled (5°C) solution of this solid (free base) in EtOH (20 ml) was treated with a saturated solution of HCl in EtOH (2 ml). The solvent was removed *in vacuo* and the solid obtained was recrystallized from CH_2Cl_2-hexane to afford (**139**) as a bright yellow crystalline solid (3.42 g, 61%); mp 121 - 125°C (dec).

^{1}H NMR ($CDCl_3$): δ 12.95 (br s, 1H, N^+HMe_2, exchanges with D_2O), 8.09 (s, 1H, aryl H-2), 8.02 (d, J = 7.9 Hz, 1H, aryl H-4), 7.64 (d, J = 7.9 Hz, 1H, aryl H-6), 7.42 (t, J = 7.9 Hz, 1H, aryl H-5), 6.33 (s, 1H, N*H*), 5.05 (s, 1H, H-4), 4.99 (septet, J = 6.3 Hz, 1H, C*H*Me_2), 4.59 (m, 2H, COOCH_2), 3.26 (m, 2H, C$H_2N^+Me_2$), 2.74 and 2.73 (two s, 3H each, N^+CH_3), 2.43 and 2.37 (two s, 3H each, C-2, C-6 CH_3), 1.27 (d, J = 6.3 Hz, 3H, CHCH_3), 1.15 (d, J = 6.3 Hz, 3H, CHCH_3).

IR (KBr): 3435 (NH), 1697 (C=O), 1533, 1350 (NO_2) cm^{-1}.

Anal. Calcd for $C_{22}H_{30}ClN_3O_6.1/2H_2O$: C, 55.40; H, 6.55; N, 8.81.
Found: C, 55.56; H, 6.60; N, 8.73.

4.4.10.2.0.0. Synthesis of 3-[2-(trimethylammonium)ethyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate iodide (140)



(140)

A solution of the free base (**139**) (2.33 g, 5.4 mmol) and iodomethane (1.32 ml, 9.27 mmol) in acetone (30 ml) was refluxed for 16 hours. The yellow residue which was obtained after removing the solvent was recrystallized from acetone-hexane to give (**140**) as a bright yellow crystalline solid (3.42g, 99%); mp 178 - 186°C.

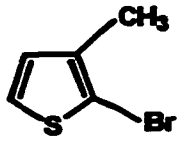
^{1}H NMR (DMSO-d_6): δ 8.93 (s, 1H, N*H*), 8.05 (t, J = 1.2 Hz, 1H, aryl H-2), 7.99 (dd, J = 7.9 Hz, J = 1.2 Hz, 1H, aryl H-4), 7.64 (d, J = 7.9 Hz, 1H, aryl H-6), 7.48 (t, J = 7.9 Hz, 1H, aryl H-5), 5.00 (s, 1H, H-4), 4.93 (septet, J = 6.3 Hz, 1H, C*H*Me$_2$), 4.42 - 4.52 (m, 2H, COOC*H*$_2$), 3.66 - 3.76 (m, 2H, C*H*$_2$N$^+$Me$_3$), 3.13 (s, 9H, N$^+$(C*H*$_3$)$_3$), 2.39 (s, 3H, C-2 C*H*$_3$), 2.30 (s, 3H, C-6 C*H*$_3$), 1.24 (d, J = 6.3 Hz, 3H, CHC*H*$_3$), 1.15 (d, J = 6.3 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3335 (NH), 1694 (C=O), 1526, 1351 (NO_2) cm^{-1}.

Anal. Calcd for $C_{23}H_{32}IN_3O_6$: C, 48.18; H, 5.62; N, 7.33.
Found: C, 48.38; H, 5.70; N, 7.20.

4.4.11.0.0.0. Synthesis of 3-[4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (144) and 3-[4,4-*bis*-(*o*-tolyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (148)

4.4.11.1.0.0. Synthesis of 2-bromo-3-methylthiophene (167)



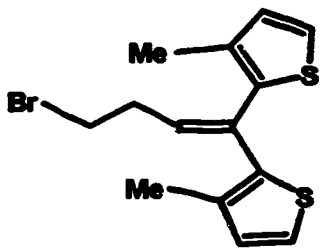
(167)

Compound (167) was prepared according to the procedure of Kellog *et al.*[228] A solution of 3-methylthiophene (4.9 g, 50 mmol) and N-bromosuccinimide (9.4 g, 52.7 mmol) in $CHCl_3$-glacial acetic acid (1:1, v/v) (60 ml) was stirred at 25°C. After 10 min., water (60 ml) was added to the solution. The organic phase was separated, washed successively with sodium carbonate (10%, w/v) (3 x 20 ml), water (3 x 20 ml) and the organic phase was dried (Na_2SO_4). Removal of the solvent *in vacuo* and distillation of the product yielded (167) as a colourless liquid (7.10 g, 80%) [bp 44 - 46°C (3 mm Hg)], bp was not reported.[228]

^{1}H NMR ($CDCl_3$): δ 7.18 (d, J = 5.5 Hz, 1H, H-5), 6.79 (d, J = 5.5 Hz, 1H, H-4), 2.21 (s, 3H, CH_3).

Compound (167) was used for the synthesis of (143).

4.4.11.1.1.0. Synthesis of 4-bromo-1,1-*bis*-(3-methyl-2-thienyl)-1-butene (143)



(143)

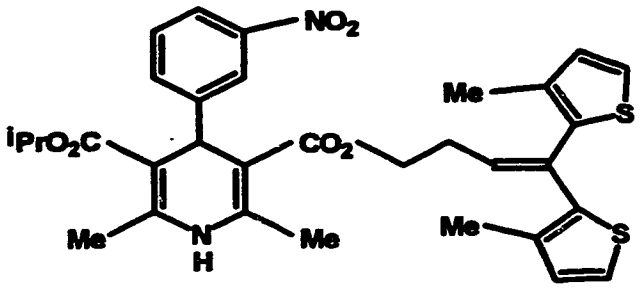
Compound (143) was prepared by a procedure reported by Knutsen *et al.*[211] A solution of (167) (7.07 g, 40 mmol) in dry ether (10 ml) was added to a stirred solution of *n*-butyllithium (2.5 M in hexane) (15.97 ml, 44 mmol) in dry ether (25 ml) at 5°C. After stirring for 15 min at 10°C, the solution mixture was cooled to -70°C and then a solution of 4-bromobutyrate (3.7 g, 19 mmol) in dry ether (10 ml) was added slowly. The reaction was allowed to proceed at -70°C for 2.5 hours prior to warming to 0°C. Cold water (30 ml) and then HCl (1 N) (15 ml) were added to the reaction mixture which was allowed to stir as the temperature was increased to 25°C. The ether layer was separated, the aqueous layer was washed with ether (3 x 15 ml) and the combined ether fractions were washed with cold water (3 x 15 ml). After drying the ether fraction (Na_2SO_4), the organic solvent was removed *in vacuo*, the residue obtained was dissolved in propan-2-ol (50 ml), and H_2SO_4 (20%, v/v) (5 ml) was added. The solution was stirred for 3 hours and the solvent was removed *in vacuo*. The residue obtained was dissolved in CH_2Cl_2 (30 ml), washed with water (3 x 25 ml), the organic phase was dried (Na_2SO_4) and the solvent was removed *in vacuo*. The residue obtained was eluted from a silica gel column using ethyl acetate-hexane (1:6, v/v) as eluent to give (143) as a yellow oil which changed colour to a purple black liquid on standing (5.25 g, 84.4%).

^{1}H NMR ($CDCl_3$): δ 7.26 (d, J = 5.1 Hz, 1H, thienyl H-5), 7.10 (d, J = 5.1 Hz, 1H, thienyl H-5), 6.88 (d, J = 5.1 Hz, 1H, thienyl H-4), 6.81 (d, J = 5.1 Hz, 1H, thienyl H-4), 6.10 (t, J = 7.0 Hz, 1H, vinyl-H), 3.46 (t, J = 7.0 Hz, 2H, C*H*$_2$Br), 2.73 (q, J = 7.0 Hz, 2H, C*H*$_2$-CH=).

IR (film): 713 (CH thiophene).

Compound (143) was used for the synthesis of (144).

4.4.11.1.2.0. Synthesis of 3-[4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (144)



(144)

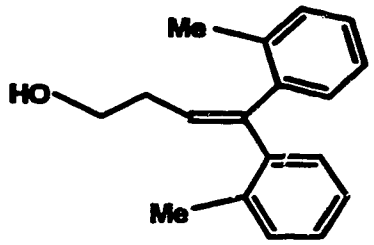
A solution of (142) (1.6 g, 4.4 mmol), (143) (1.7 g, 5.2 mmol) and potassium carbonate (1.23 g, 8.8 mmol) in dry DMF (20 ml) was stirred at 25°C for 120 hours. HCl (6 N) was then added until the pH of the solution was 1 during which unreacted (142) (0.5 g) precipitated. After filtration, this solution was evaporated *in vacuo* to remove as much DMF as possible. The residue obtained was dissolved in ethyl acetate (20 ml) and was washed with water (3 x 25 ml). After drying the organic layer (Na_2SO_4), the solvent was removed *in vacuo* and the residue obtained was eluted from a silica gel column using ethyl acetate-hexane (2:5, v/v) as eluent. Unreacted (143) (0.8 g) eluted first. The product (144) eluted next as a yellow foam that was recrystallized from isopropyl ether-hexane as a light yellow crystalline solid which turned to a purple black colour on standing (0.72 g, 27%); mp 105 - 107°C.

^{1}H NMR ($CDCl_3$): δ 8.13 (t, J = 1.6 Hz, 1H, aryl H-2), 7.94 (dd, J = 7.9 Hz, J = 1.6 Hz, 1H, aryl H-4), 7.54 (d, J = 7.9 Hz, 1H, aryl H-6), 7.28 (t, J = 7.9 Hz, 1H, aryl H-5), 7.22 (d, J = 5.1 Hz, 1H, thienyl H-5), 7.06 (d, J = 5.1 Hz, 1H, thienyl H-5), 6.85 (d, J = 5.1 Hz, 1H, thienyl H-4), 6.76 (d, J = 5.1 Hz, 1H, thienyl H-4), 6.06 (s, 1H, N*H*), 5.93 (t, J = 6.8 Hz, 1H, vinyl-H), 5.08 (s, 1H, H-4), 4.95 (septet, J = 6.3 Hz, 1H, C*H*Me$_2$), 4.14 (t, J = 6.8 Hz, 2H, COOC*H*$_2$CH$_2$), 2.44 (q, J = 6.8 Hz, 2H, C*H*$_2$-C=), 2.36 and 2.34 (two s, 3H each, C-2 and C-6 C*H*$_3$), 2.03 and 1.95 (two s, 3H each, thienyl C*H*$_3$), 1.25 (d, J = 6.3 Hz, 3H, CHC*H*$_3$), 1.10 (d, J = 6.3 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3376 (NH), 1691 (C=O), 1528, 1349 (NO_2), 712 (CH, thiophene) cm^{-1}.

Anal. Calcd for $C_{32}H_{34}N_2O_6S_2$: C, 63.35; H, 5.65; N, 4.62.
Found: C, 63.65; H, 5.65; N, 4.55.

4.4.11.2.0.0. Synthesis of 4,4-*bis*-(*o*-tolyl)-3-butenol (146)



(146)

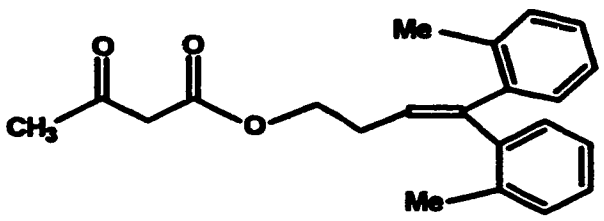
To a stirred ice-cooled solution of 4-butyrolactone (2.22 g, 25.8 mmol) in dry diethyl ether (50 ml) was added *o*-tolylmagnesium bromide (2.0 M in diethyl ether) (30 ml, 60 mmol). During this additon, a white precipitate was formed which on further stirring gave rise to a cloudy solution. Stirring was continued for 2 hours at 25°C prior to the addition of saturated NH_4Cl (50 ml) at 5°C. The precipitate was then filtered and the filtrate was extracted with ethyl acetate (3 x 20 ml). The combined organic extracts were dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield a white solid which was then dissolved in a solution of EtOH and HCl (4 N) (1:1, v/v) (100 ml). This solution was heated at reflux for 6 hours and the solvent was removed *in vacuo*. Water (50 ml) was added to the residue. Extraction with ethyl acetate (3 x 50 ml), drying the extracts (Na_2SO_4) and evaporation of the solvent yielded a dark brown residue which on elution from a silica gel column using ethyl acetate-hexane (1:2, v/v) as eluent yielded (146) as a yellow oil (4.76 g, 73%).

^{1}H NMR ($CDCl_3$): δ 7.09 - 7.18 (m, 8H, aryl-H), 5.82 (t, *J* = 6.9 Hz, 1H, vinyl-H), 3.72 (t, J = 6.9 Hz, 2H, C*H*$_2$OH), 2.35 (q, J = 6.9 Hz, 2H, C*H*$_2$-C=), 2.30 (s, 3H, aryl-C*H*$_3$), 2.13 (s, 3H, aryl-C*H*$_3$), 1.38 (br s, OH, exchanges with D_2O).

IR (film): 3120 - 3640 (br) (OH), 1048 (C-O) cm^{-1}.

Compound (146) was used immediately for the synthesis of (147).

4.4.11.2.1.0. Synthesis of 4,4-*bis*-(o-tolyl)-3-butenyl acetoacetate (147)



(147)

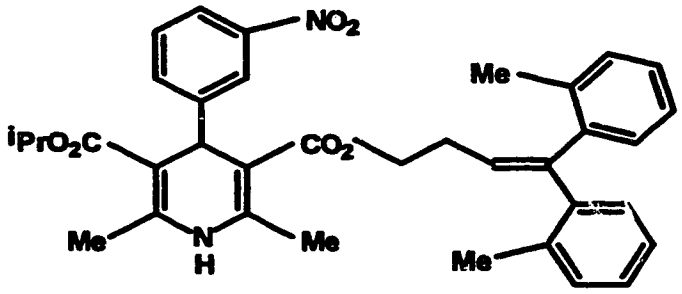
The title compound (147) was prepared according to Procedure A by reaction of 4,4-*bis*-(*o*-tolyl)-3-butenol (146) (1.02 g, 4 mmol), diketene (0.44 g, 5.2 mmol) and triethylamine (0.5 ml, 9.2 mmol). The reaction product was purified by silica gel column chromatography using ethyl acetate-hexane (1:2, v/v) as eluent to afford (147) as a light yellow liquid (1.19 g, 84%).

^{1}H NMR ($CDCl_3$): δ 7.08 - 7.27 (m, 8H, aryl-H), 5.79 (t, J = 6.9 Hz, 1H, vinyl-H), 4.23 (t, J = 6.9 Hz, 2H, COOCH_2), 3.45 (s, 2H, COCH_2COO), 2.44 (q, J = 6.9 Hz, 2H, CH_2-CH=C), 2.29 (s, 3H, aryl-CH_3), 2.27 (s, 3H, aryl-CH_3), 2.13 (s, 3H, CH_3CO).

IR (film): 1745 (C=O, ester), 1721 (C=O, ketone) cm^{-1}.

Compound (147) was used for the synthesis of (148).

4.4.11.2.2.0. Synthesis of 3-[4,4-*bis*-(*o*-tolyl)-3-butenyl] 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (148)



(148)

Procedure C was employed for the synthesis of (148). Thus, condensation of 3-nitrobenzaldehyde (0.49 g, 3.24 mmol) with (147) (1.09 g, 3.24 mmol) and isopropyl 3-aminocrotonate (0.46 g, 3.24 mmol), and purification of the product by silica gel

column chromatography using ethyl acetate-hexane (1:4, v/v) as eluent afforded (**148**) as a yellow crystalline solid after recrystallization from CH_2Cl_2-hexane (1.01 g, 49%); mp 157 - 159°C.

^{1}H NMR ($CDCl_3$): δ 8.10 (t, J = 1.8 Hz, 1H, nitrophenyl H-2), 7.92 (d, J = 8.0 Hz, 1H, nitrophenyl H-4), 7.60 (d, J = 8.0 Hz, 1H, nitrophenyl H-6), 7.22 (t, J = 8.0 Hz, 1H, nitrophenyl H-5), 7.02 - 7.20 (m, 8H, *o*-tolyl-H), 5.62 - 5.67 (m, 2H, N*H* and vinyl-H), 5.06 (s, 1H, H-4), 4.95 (septet, 1H, J = 6.2 Hz, C*H*Me$_2$), 4.10 (t, J = 6.6 Hz, 2H, COOC*H*$_2$), 2.30 - 2.42 (m, 8H, C-2 and C-6 C*H*$_3$ and C*H*$_2$-CH=), 2.20 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.08 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

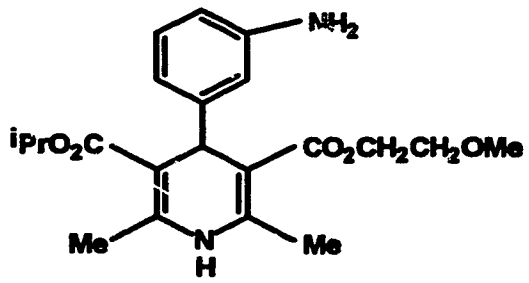
IR (KBr): 3353 (NH), 1696, 1664 (C=O), 1532, 1359 (NO_2) cm^{-1}.

Anal. Calcd for $C_{36}H_{38}N_2O_6$: C, 72.71; H, 6.44; N, 4.71.

Found: C, 72.37; H, 6.30; N, 4.83.

4.4.12.0.0.0. Syntheses of 3-(2-methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-dimethylaminophenyl)-3,5-pyridinedicarboxylate (152) and 3,5-diisopropyl 1,4-dihydro-2,6-dimethyl-4-(3-dimethylaminophenyl)-3,5-pyridinedicarboxylate (153)

4.4.12.1.0.0. Synthesis of 3-(2-methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-aminophenyl)-3,5-pyridinedicarboxylate (158) (Procedure J)



(158)

A solution of nimodipine (**38**) (0.41 g, 0.98 mmol) in EtOH (30 ml) was added to a pressure bottle containing palladium-on-carbon (10%) (0.1 g). This solution and its contents was shaken under an atmosphere of hydrogen (55 psi) for 2 hours. Filtration of

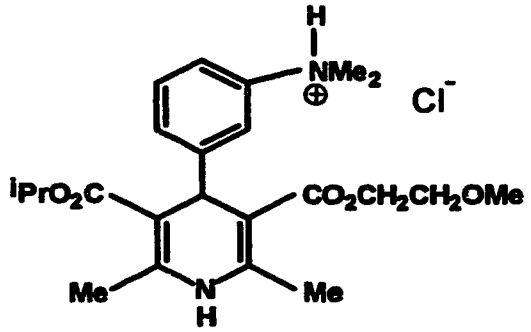
the palladium catalyst, and evaporation of the solvent *in vacuo* afforded (158) as a grey coloured oil (0.37 g, 95%).

^{1}H NMR ($CDCl_3$): δ 6.98 (t, J = 7.7 Hz, 1H, aryl H-5), 6.70 (d, J = 7.7 Hz, 1H, aryl H-6), 6.65 (t, J = 1.9 Hz, 1H, aryl H-2), 6.46 (dd, J = 7.7 Hz, J = 1.9 Hz, 1H, aryl H-4), 5.70 (br s, 1H, dihydropyridyl N*H*), 4.94 - 4.98 (m, 2H, C*H*Me$_2$ and H-4), 4.10 - 4.26 (m, 2H, COOC*H*$_2$), 3.53 - 3.59 (m, 4H, C*H*$_2$OMe and NH_2, exchanges with D_2O), 3.37 (s, 3H, OC*H*$_3$), 2.31 (s, 6H, C-2 and C-6 C*H*$_3$), 1.24 (d, J = 6.3 Hz, 3H, CHC*H*$_3$), 1.14 (d, J = 6.3 Hz, 6H, CHC*H*$_3$).

IR (film): 3338 (NH), 1648 (C=O).

Compound (158) was used immediately for the synthesis of (152).

4.4.12.1.1.0. Synthesis of 3-(2-methoxyethyl) 5-isopropyl 1,4-dihydro-2,6-dimethyl-4-(3-dimethylaminophenyl)-3,5-pyridinedicarboxylate hydrochloride (152) (Procedure K)



(152)

A procedure for the reductive-methylation of primary amines reported by Kim *et al*[229] was used for the preparation of (152). To a stirred solution of (158) (2.73 g, 7.03 mmol) and formaldehyde (37%, w/v) (1.72 ml, 21.0 mmol) in MeOH (25 ml) was added a suspension of sodium cyanoborohydride (0.44 g, 7.03 mmol) and zinc chloride (0.48 g, 3.5 mmol) in MeOH (25 ml). The reaction was allowed to proceed at 25°C for 18 hours before addition of NaOH (0.2 M) (50 ml). The organic solvent was evaporated *in vacuo* before extracting the aqueous residue with ethyl acetate (3 x 25 ml). The combined ethyl acetate extracts were dried (Na_2SO_4) and the solvent was evaporated. The residue

obtained was purified by silica gel column chromatography using ethyl acetate-hexane (1:2, v/v) as eluent to afford a yellow oil which was the free base of (152). This oil was then dissolved in EtOH (50 ml) and a saturated solution of HCl in EtOH (40 ml) was added at 0°C. This solution was stirred for 15 min, the solvent was removed *in vacuo* and the residue obtained was recrystallized from EtOH to yield (152) as a white crystalline solid (0.62 g, 19.5%); mp 209 - 210°C (dec).

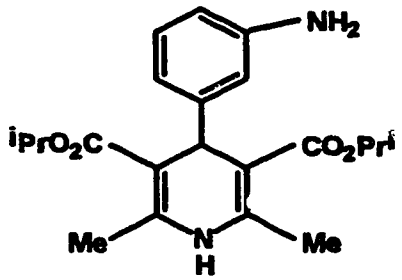
^{1}H NMR (DMSO-d_6): δ 8.96 (s, 1H, N*H*), 6.80 - 7.64 (br m, 4H, aryl-H), 4.87 (s, 1H, H-4), 4.81 (septet, J = 6.2 Hz, 1H, C*H*Me$_2$), 4.04 - 4.14 (m, 2H, COOC*H*$_2$), 3.52 (t, J = 4.7 Hz, 2H, CH$_2$C*H*$_2$OMe), 3.26 (s, 3H, OC*H*$_3$), 3.01 (s, 6H, N$^+$C*H*$_3$), 2.27 and 2.26 (two s, 3H each, C-2 and C-6 C*H*$_3$), 1.18 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.05 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3471, 3198 (NH), 1689 (C=O) cm^{-1}.

Anal. Calcd for $C_{23}H_{33}ClN_2O_5$: C, 60.99; H, 7.34; N, 6.18.

Found: C, 60.55; H, 7.24; N, 6.08.

4.4.12.2.0.0. Synthesis of 3,5-diisopropyl 1,4-dihydro-2,6-dimethyl-4-(3-aminophenyl)-3,5-pyridinedicarboxylate (157)



(157)

Compound (157) was synthesized according to Procedure J by reduction of (156) (0.44 g, 1.1 mmol) with 10% palladium-on-carbon (0.1 g) and hydrogen gas at 55 psi to afford (157) as a yellow foam (0.39 g, 99%).

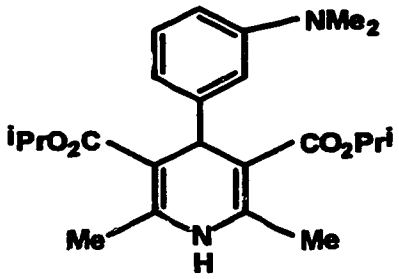
^{1}H NMR ($CDCl_3$): δ 6.98 (t, J = 7.7 Hz, 1H, aryl H-5), 6.70 (d, J = 7.7 Hz, 1H, aryl

H-6), 6.62 (t, J = 1.7 Hz, 1H, aryl H-2), 6.46 (dd, J = 7.7 Hz, J = 1.7 Hz, 1H, aryl H-4), 5.59 (s, 1H, dihydropyridyl N*H*), 4.90 - 5.59 (m, 3H, C*H*Me$_2$ and H-4), 3.30 - 3.70 (br s, 2H, N*H*$_2$, exchanges with D_2O), 2.31 (s, 6H, C-2 and C-6 C*H*$_3$), 1.25 (d, J = 6.2 Hz, 3H, CHC*H*$_3$), 1.15 (d, J = 6.2 Hz, 3H, CHC*H*$_3$).

IR (KBr): 3343 (NH), 1645 (C=O) cm^{-1}.

Compound (**157**) was used immediately for the synthesis of (**153**).

4.4.12.2.1.0. Synthesis of 3,5-diisopropyl 1,4-dihydro-2,6-dimethyl-4-(3-dimethylaminophenyl)-3,5-pyridinedicarboxylate (153)



(153)

Procedure K was used for the synthesis of (**153**) by reaction of (**157**) (3.11 g, 8.35 mmol) with formaldehyde (37%, w/v) (2.04 ml, 25 mmol), sodium cyanoborohydride (0.53 g, 8.35 mmol) and zinc chloride (0.57 g, 4.17 mmol). The product was purified by silica gel column chromatography using ethyl acetate-hexane (1:2, v/v) as eluent to yield (**153**) which was recrystallized from CH_2Cl_2-hexane as a yellow crystalline solid (1.69 g, 50%); mp 136 - 139°C.

^{1}H NMR ($CDCl_3$): δ 7.09 (t, J = 7.9 Hz, 1H, aryl H-5), 6.77 (br m, 1H, aryl H-6), 6.68 (br m, 1H, aryl H-2), 6.55 (br m, 1H, aryl H-4), 5.53 (br s, 1H, N*H*), 4.97 (m, 3H, two C*H*Me$_2$ and H-4), 2.91 (s, 6H, NC*H*$_3$), 2.33 (s, 6H, C-2 and C-6 C*H*$_3$), 1.25 (d, J = 6.2 Hz, 6H, CHC*H*$_3$), 1.16 (d, J = 6.2 Hz, 6H, CHC*H*$_3$).

IR (KBr): 3356 (NH), 1692, 1648 (C=O) cm^{-1}.

Anal. Calcd for $C_{23}H_{32}N_2O_4.1/4H_2O$: C, 68.21; H, 8.09; N, 6.92.

Found: C, 68.30; H, 7.81; N, 7.06.

4.5.0.0.0.0. High performance liquid chromatography (HPLC) assays

4.5.1.0.0.0. HPLC assay for felodipine (39)

The mobile phase consisted of acetonitrile:water (1:1, v/v) and a flow rate of 1.0 ml / min was employed.

The internal standard (I.S.) for this assay was prepared by dissolving 3-(2-hydroxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (**102**) in EtOH (7.5 mg / 5 ml) (solution A). A standard solution for felodipine was also prepared (12.0 mg / 5 ml EtOH) (solution B). Five standard solutions (C, D, E, F and G) were prepared by delivering 16, 32, 48, 64, and 80 µl, respectively of solution B to 5 standard flasks each containing 20 µl of I.S. (solution A). The volume of each flask was adjusted to 10 ml with EtOH. A calibration curve was prepared by injecting 15 µl of each standard solution C, D, E, F and G. The mobile phase consisted of acetonitrile-water (1:1, v/v) with a flow rate of 1 ml/min. Using a 3.9 x 150 mm reverse phase C_{18} column and UV detection at 350 nm, the retention times were 2.7 and 10.0 min for the internal standard and felodipine respectively. A linear calibration curve for felodipine was obtained in this way. (correlation coefficient 0.9974)

The analyses procedure for biological samples obtained from rat plasma and brain homogenate were the same. To each sample (500 µl) was added 1 µl of I.S. (solution A), and this solution was centrifuged for 5 min (15,000 rpm). Then, 15 µl of the supernatant liquid was removed for HPLC analyses. The HPLC conditions employed were similar to the calibration described previously. The data were quantitated using Millennium 2010 software employing the calibration curve previously constructed.

4.5.1.1.0.0. HPLC assay for determining the purity of felodipine (39)

The felodipine products synthesized using Methods A and B (3.1.2.0.0.0.) were subjected to HPLC analyses. The HPLC conditions employed were similar to those used in the *in vitro* and *in vivo* assays but without using the internal standard. Thus, a solution of the sample was prepared by dissolving 4.5 mg of the product in 5 ml of ethanol. The retention times for the dimethyl ester (**78**), felodipine (**39**) and the diethyl

ester (79) were found to be 7.0, 10.0 and 14.4 minutes respectively. The relative amount of (78), (39) and (79) was calculated from the respective areas under the respective peaks.

4.5.2.0.0.0. HPLC assay for 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (118)

The mobile phase consisted of acetonitrile-phosphate buffer (NaH_2PO_4, 0.015 M) using gradient mode elution starting from 38% acetonitrile and going to 100% of acetonitrile at 20 min. The flow rate started at 1 ml / min until 2 min, when it was changed linearly to a rate of 5 ml / min at 20 min.

The internal standard (I.S.) was prepared by dissolving 3-(2-methoxyethyl) 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (165) (1.1 mg / 5 ml DMSO) (solution H). A standard solution containing 2-hydroxyethyl DHP (102), the quaternary pyridinium salt (115) and the CDS-DHP (118) was prepared by dissolving 7.5 mg, 11.2 mg, and 11.0 mg of each compound, respectively in DMSO (5 ml) and the final volume was adjusted to 10 ml by adding more DMSO. (solution I). Five standard solutions (J, K, L, M and N) were prepared by delivering 10, 20, 30, 40 and 50 µl of solution I to five volumetric flasks each containing 100 µl of I.S. (solution H). The final volume of each flask was adjusted to 5 ml by adding DMSO.

Calibration, performed by injecting 15 µl of each soluiton J, K, L, M and N, provided retention times for (102), (115), I.S. and (118) of 5.8, 7.3, 9.8 and 10.6 min, respectively. A linear calibration curve for each compound was obtained (correlation coefficient : 0.9974 - 0.9997).

The HPLC analyses protocol for analysis of the biological samples (rat plasma and brain homogenate) was similar to the procedure described for the felodipine assay. 10 µl of the internal standard solution H was added to 500 µl of the sample before analysis.

4.6.0.0.0.0. *In vitro* incubation studies employing felodipine (39) and 3-[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (118)

Male Sprague-Dawley rats weighing 250 - 300 g were purchased from the Health Sciences Laboratory Animal Services Facility, University of Alberta. The rats were sacrificed by inhalation of CO_2 released from dry ice. Blood samples were obtained by heart puncture and collected in heparinized tubes. The entire brain was removed after incision of the skull.

Rat plasma was obtained by centrifuging whole blood (10 ml) at 3000 rpm for 15 min. The supernatant light yellow plasma was removed using a pipette. Rat whole brain was homogenized with phosphate buffer (pH = 7.4) (1:4, w/v; brain:phosphate buffer) by using a Con-Torque power unit, Eberback Corporation. The homogenate was then filtered through filtered paper (Whatman No.1).

Felodipine (solution B) (120 µl) was added to 3 sample tubes each containing rat plasma (2 ml) or brain homogenate (2 ml). The solution was shaken in an incubator or water bath at 37°C. At selected time intervals (0.08, 0.25, 0.5, 0.75, 1, 2, 3, 6, 22, 31, 46, 117 and 119 hours), a 100 µl aliquot was withdrawn and added to 400 µl of ice-cold CH_3CN . The sample was then shaken and stored at -20°C in the dark until subjected to HPLC analysis.

Similar *in vitro* incubation studies were performed using the CDS compound (118). 40 µl of a standard solution of (118) (14.2 mg / 5 ml DMSO) was used for each incubation sample of rat plasma or brain homogenate (2 ml). A 100 µl aliquot was withdrawn at selected time intervals (6, 12, 24, 37, 48, 60, 72, 84 and 96 hours for plasma samples and 0.17, 0.3, 0.58, 0.83, 1, 2, 3, 4, 5, 6, 7 and 8 hours for brain homogenate samples) and added into 400 µl of cold CH_3CN solution.

4.7.0.0.0.0. *In vivo* rat distribution studies for felodipine (39) and 3[2-(1-methyl-1,4-dihydropyridyl-3-carbonyloxy)ethyl] 5-methyl 1,4-dihydro-2,6-dimethyl-4-(2,3-dichlorophenyl)-3,5-pyridinedicarboxylate (118)

Male Sprague-Dawley rats weighing 250 - 300 g were used in these studies. A solution of felodipine was prepared by dissolution in DMSO:saline (0.9%, w/v) (85:15, v/v) to provide a concentration in the 14 - 15 mg/ml range. A 2.5 mg/kg dose of felodipine was administered by tail-vein injection. The rat was temporarily restrained at the time of injection, and then placed in a cage with free access to food and water ad libitum. Three rats were sacrificed for each selected time (5, 15, 30, 45, 60 and 90 min) after dosing. Rats were sacrificed by inhalation of CO_2 generated from dry ice. Whole blood samples were collected by cardiac puncture and placed in heparinized tubes. Blood (1 ml) was withdrawn from this tube and it was added to 2 ml of a solution of CH_3CN-DMSO (94:6, v/v). The blood sample was then stirred and centrifuged (4,000 rpm) for 10 minutes and 500 µl of the supernatant liquid obtained was pipetted into a sample tube which was stored at - 20°C in the dark prior to HPLC analysis.

Rat whole brain was removed following skull incision. This brain tissue was washed with saline (0.9 %, w/v) (20 ml), dried on a filter paper and was then homogenized in a tube containing CH_3CN-DMSO (94:6, v/v) (1:2, w/v; brain:organic solvent). Centrifugation at 4,000 rpm was carried out for 10 min, and the supernatant liquid was withdrawn (500 µl) into a sample tube which was stored at - 20°C in the dark prior to HPLC analysis.

The CDS (**118**) was administered to rats at a dose of 15 mg/kg by tail-vein injection. The vehicle for this study was DMSO-saline (0.9%, w/v) (85:15, v/v) and the concentration of the test-drug solution was in the range of 25 - 26 mg / ml. The rats were sacrificed at 0.08, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 96 hours after drug administration. Three rats were used for each time interval and the blood and brain samples collected were treated similarly as in the felodipine studies.

4.8.0.0.0.0. Determination of Partition coefficients (Kp)

Octanol-phosphate buffer partition coefficient (Kp) values were determined by the method of Fujita *et al.*[230] *n*-Octanol (500 ml) was purified by successive washing with dilute H_2SO_4 (1 N) (3 x 30 ml), NaOH (1 N) (3 x 30 ml) and water (3 x 100 ml) prior to distillation *in vacuo* at 80°C (3 mm Hg) .

n-Octanol and aqueous phosphate buffer (pH = 7.4) were mutually saturated, before use for Kp determinations, by stirring equal volumes of each component at 25°C for 16 hours. After standing for 1 hour, the two layers were separated.

Standard solutions were prepared by dissolving accurately weight amount (7.5 - 10 mg) of the test compound in 5 ml of *n*-octanol (except for **(140)** which was dissolved in the phosphate buffer) (solution A). Known volumes of this solution were then diluted in volumetric flasks to give five standard solutions. Each solution was then analyzed by UV spectrometry and a calibration curve was prepared by plotting an absorbance versus concentration curve. The wavelengths for UV analyses were determined from the UV spectra, the λ_{max}, of each test compound (λ_{max} varies from 345 - 360 nm).

A known volume of solution A (150 µl) was then pipetted into a glass tube containing *n*-octanol (4 ml) and phosphate buffer (40 ml). The tube with its contents was then shaken for 1 hour at 25°C. After standing for 15 min, the tube was centrifuged for 10 min (3,600 rpm) to completely separate the two layers. The *n*-octanol layer was removed for UV analysis except for **(140)** where the aqueous layer was analyzed. The concentration of the test compound in the *n*-octanol layer was then determined from the calibration curve. The difference in concentration before and after partitioning gives the amount of the test compound that was partitioned in the aqueous buffer. The partition coefficient was calculated from the equation Kp = concentration of test compound in octanol / concentration of test compound in phosphate buffer.

5.0.0.0.0.0. REFERENCES

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